LEAF-SCALD

A BACTERIAL DISEASE OF SUGAR CANE

BY

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PLANT PATHOLOGIST TO

THE COLONIAL SUGAR REFINING COMPANY, LIMITED

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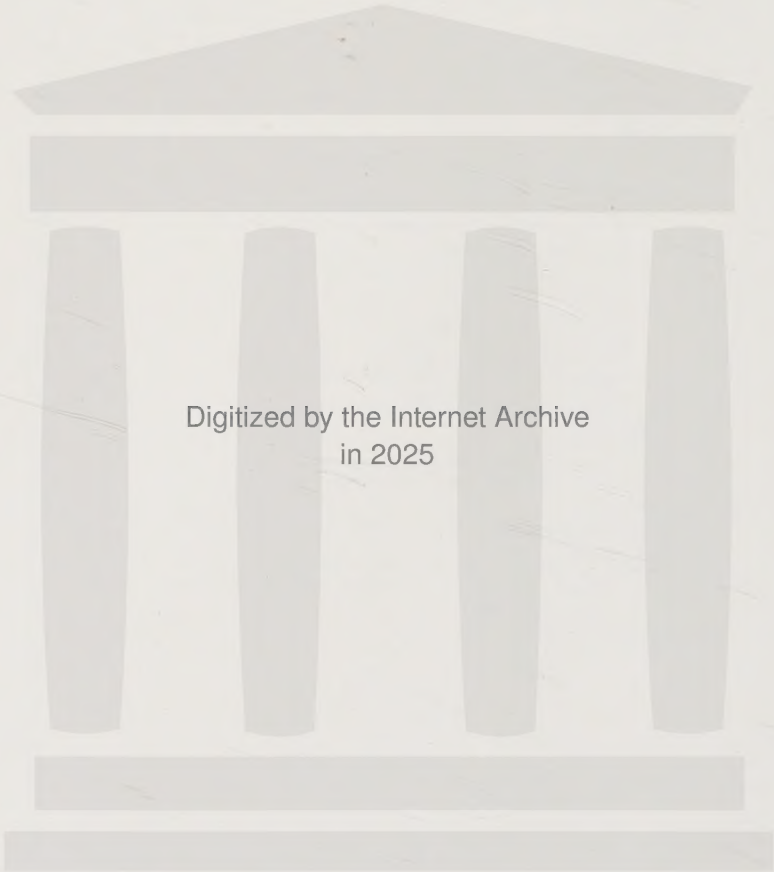
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LEAF-SCALD

A BACTERIAL DISEASE OF SUGAR CANE.

Introduction.

The paper presented in the following pages was prepared in draft form in 1923, dealing with investigations made chiefly at Broadwater Mill, Richmond River, N.S.Wales, between August, 1919, and December, 1922. Its publication was first delayed several months awaiting the arrival and translation of G. Wilbrink's paper on the gum disease of Java (8)*, and von Wolzogen Kühr's paper on sereh disease (3), both of which it seemed desirable to consult before inserting a discussion of the relationship of leaf-scald to these diseases. The crowding of other work then caused the further delay which has occurred since.

A study of the earlier literature made during the course of the investigations indicated that both the sereh and gum diseases of Java are distinct from leaf-scald, although showing a resemblance in certain respects. Groenewege's investigations (1 and 2) particularly seemed to establish the gum disease as different, by his finding that it is not propagated by infected cuttings, to mention only one of the important distinctions. Correspondence with the Director of the Experiment Station at Pasoeroean then showed the Java gum disease to be distinct from Cobb's gumming disease, but indicated that it is merely a phase of sereh. His letter of 27/7/20 enclosed a report by von Wolzogen Kühr, who then claimed to have indications that " ' Sereh ' is the chronical form and ' gomziekte ' the severe, and often deadly attack for young plants, by *Bacillus herbicola vascularum* . . . . ."

* Reference is made by number to " Literature Cited," p. 80.

However, the fallacies of these conclusions were clearly demonstrated by Wilbrink (8). She definitely proved the cause of the Java gum disease to be a bacterium, which is quite distinct from the one isolated by Groenewege (1), and erroneously described by him as *Bact. vascularum*, Cobb, as also from the true *Bact. vascularum*, the cause of Cobb's gumming disease. She reversed Groenewege's conclusions also on other points, particularly on those which seemed to establish the Java gum disease to be distinct from leaf-scald.

She also definitely showed sereh disease to be distinct, by finding that her gum disease bacterium does not occur in sereh diseased plants, and does not cause sereh to develop in susceptible varieties inoculated with it. This bacterium is also quite distinct from *Bacterium herbicola*, which Kühr (3) found constantly associated with sereh. The latter also compared these two diseases in the course of his extensive investigations and confirmed Wilbrink's findings generally.

Further, Wilbrink's account of the Java gum disease agrees with the writer's observations on leaf-scald in Australia, not only in all essential features, but even in minor details in the great majority of cases. Both of us find the same fine whitish streaks on the leaves to be the chief symptom for recognition in the field, having traced these streaks through practically all the same variations in appearance. Similar agreement is also found in regard to the rapidly wilting phase ; the chronic phase, with numerous side-shoots and white chlorotic leaves ; the reddened vascular bundles ; stagnation in growth ; and the distribution of the bacteria in the different organs of the plant, in which they are usually confined to the xylem vessels of the vascular system. Much the same difficulties were encountered in isolating the bacteria from the plant tissues and in cultivating them. It may be mentioned here that the writer even performed his first inoculation tests with a non-pathogenic bacterium similar to that described by Wilbrink as the pseudo gum-disease bacterium, and, of course, similarly discarded it as the wrong organism. As regards the true pathogen, the rate of growth and cultural characters observed generally are in close agreement. However, the writer was unable to cultivate the organism as successfully as Wilbrink, who apparently devised more suitable media for this purpose, and thus carried the study of its cultural characters much further. Next, in the inoculation tests, each found young parts of stalks, immature leaves in the apical spindle, and cuttings ready to be planted, to be susceptible to wound infection. But mature leaves and roots proved non-

susceptible, or showed at most transitory effects. Both also clearly showed that seed cane from diseased stock will produce diseased crops, and that knife-infection is an important further means of dissemination. In short, our observations are in remarkably close agreement almost throughout.

The more definite points upon which full agreement apparently does not obtain, as gathered by a critical reading of Wilbrink's paper, are compared in the following tabulation :—

APPARENT DIFFERENCES BETWEEN THE JAVA GUM DISEASE ("GOMZIEKTE") AND THE AUSTRALIAN LEAF-SCALD.

	Gum Disease. Wilbrink.	Leaf-scald. North.
Symptoms : 1. Striped appearance of leaves.	Severely attacked leaves sometimes show a completely green and white striped appearance, as a result of the large number of stripes.	A green and white striped appearance frequently results from etiolation, but leaf-streaks have never been seen numerous enough to produce this appearance.
2. Rind of stems.	The young internodes, which are not yet pigmented, show more or less red coloured bands which may even have grown rotten. The latter, however, rarely occurs.	No red coloured bands have been observed. But pigmentation of the young internodes develops in an irregular, unhealthy manner, sometimes suggesting strokes or bands.
3. Growing points.	In gum-diseased stalks there is a strong red-colouring of the growing points, in the top growing point as well as those in the side-shoots. This red-colouring, if the cut-open tissues remain exposed to the air, oxidises quickly to foul-brown. This phenomenon, however, can be observed with all cane stalks, in more or less degree, when the water supply is insufficient. The red-colouring is not limited to the growing points, but the tissues below sometimes also show a more or less pink tint.	No such strong red- colouring of the growing points has been observed. Those of diseased stalks are slightly more coloured (orange yellow) than those of sound stalks and oxidise more rapidly on exposure to the air. In this respect, diseased stalks closely resemble stalks affected by insufficient supply of water.

	Gum Disease. Wilbrink.	Leaf-scald. North.
4. Rotting of roots.	When the attack is very severe, the whole root system may be rotten, so that the plants can be pulled out of the soil quite easily.	Diseased stools which can be pulled out of the soil easily, owing to the root systems being rotten, have never been found. Except that when diseased cuttings are planted, very few roots develop, and these soon die and rot, the plant rarely surviving more than two or three months ; these plants can easily be pulled out.
5. Vascular bundles of leaves infected.	Smaller vascular bundles without lysigenous spaces, and the transverse junctions between vascular bundles are rarely infected.	Smaller vascular bundles are frequently infected, and masses of bacteria have been seen in the transverse junctions.
Causal Organisms. 6. Size in cane tissues.	Nearly 1μ long x little less than 0.5μ broad, measured alive in water. Slightly smaller when stained with carbol-fuchsin.	1.0μ long x 0.2μ broad, stained with carbol-fuchsin.
7. Form in cultures.	Remains constant, or nearly so. Is not liable to degeneration. A culture, ten months old, on sucrose peptone agar was still alive.	Shows great variability. Produces curved and enlarged involution forms, sometimes twisted threads. Usually degenerates and disintegrates, dying out in a month or so. Occasionally cultures two months, and even three months old were still alive.
8. Growth in peptonised meat-extract.	Grows freely.	Almost fails to grow.

A few other apparent differences were noted, which seem to depend rather on interpretation or mode of expression than on an actual difference in the features observed. For example, the

writer describes bacterial cavities, whereas Wilbrink apparently considers that bacterial cavities do not occur. Yet she describes cavities in the parenchyma filled with air and never with bacterial slime or gum ; also the phloem tissues and xylem-parenchyma flooded with bacteria in rare cases, when these parts are becoming rotten. Her lengthy description of these phenomena seems to correspond closely with what the writer observed and called bacterial cavities.

In view of the remarkably close agreement found in the great mass of the observations, these few minor differences seem insufficient to establish the two diseases as distinct. Their explanation probably lies partly in the fact that the two diseases were investigated in entirely different varieties of sugar cane, grown under widely different environmental conditions, and partly in the different methods of technique employed, or in errors of observation. Differences Nos. 7 and 8 in the above table, and the superior growth displayed by the bacterium in Wilbrink's cultures generally, may possibly have been due to the brand of peptone she used being better suited to its requirements than that used by the writer. (*See* p. 42).

Thus the conclusion is arrived at that the Javanese gum disease and the Australian leaf-scald are in all probability identical. Even though some or all of the differences should prove to be valid, the two diseases, though not identical, must still be very closely related, much closer than, say, Cobb's gumming to the Javanese gum disease.

Wilbrink has made important findings beyond the writer, in the study of the cultural characters of the organism as already mentioned, and in finding that the roots are invaded by the parasite and rot.

On the other hand, the writer has demonstrated that there are other important means of dissemination, besides those found by Wilbrink, although he has not determined their precise nature ; also, that the selection of healthy planting material is seriously interfered with by the ability of the parasite to exist for a long time in a latent condition in cane of healthy appearance. He, therefore, considers that the control measures proposed by Wilbrink will prove inadequate. Particularly, an attempt to keep bibit nurseries free of gum-disease by pulling out and destroying all diseased plants, is likely to prove unsuccessful and to lead to disappointment.

The present paper thus covers much of the same ground as Wilbrink's, although it was originally written in the belief that

a previously undescribed disease was being dealt with. Its original form is being adhered to, as thus affording better confirmation of Wilbrink's work under the circumstances. Also, the reader will thus appreciate the wasted effort and disappointment which have resulted from working without comprehensive library facilities.

1. Type.

Leaf-scald is the name popularly applied to a destructive disease of sugar cane in Australia and Fiji. It is a disease of the vascular system, closely resembling Cobb's gumming disease (*Bacterium vascularum* (Cobb), Grieg-Smith) in its mode of attack upon the plant. The latter, which was fully investigated by Erwin F. Smith (7), following other investigators, served as a convenient standard of comparison, the two diseases occurring together in our fields and having been studied side by side throughout the investigations.

They are quite distinct from each other, as the leaf-scald organism occurs in the cane tissues as a minute rod appreciably smaller, and especially more slender than *B. vascularum*. Also, the former does not grow readily in ordinary culture media, but requires media specially adapted to it for successful cultivation. Even then its growth is slower and more feeble than that of the latter. It does not produce gum (slime) either in cultures or in the cane tissues, and many weird enlarged involution forms are produced in artificial cultures.

Their symptoms also serve to separate the two diseases in the field. Both produce leaf-streaks as their most useful symptom for diagnostic purposes. But, with leaf-scald, the streak may be seen as soon as the leaf unfolds and turns green, or even in an immature white leaf unfolded by hand. It is at first a straight, narrow, well defined, creamy or greyish-white streak, often extending throughout the length of leaf-blade and leaf-sheath. Whereas those of gumming always present a distinct yellowish tint, they rarely appear in young leaves, they are broader, not so straight, have an irregular outline, and are usually much shorter, rarely extending the whole length of the leaf.

When a stalk is cut, no gum oozes in the case of leaf-scald, but, instead, the cut surface presents a peculiar dry appearance.

2. History and Distribution.

Mahona, a cane variety introduced from New Guinea in 1896 (No. 22 of Tryon's collection), was found to produce exceptionally high quality, quick growth, and heavy crops. But, owing to "too

early maturing and dying off," it came to be regarded as unsuited to tropical districts (North Queensland and Fiji). This popular conception was strengthened by its success especially as a one-year-old cropper in the sub-tropical Northern River districts of N.S.W., where crops are mostly harvested at two-years-old.

Many seedlings of Mahona parentage were raised in Queensland and Fiji by the late Mr. James Clark, in an endeavour to reproduce its good qualities without the objectionable one of "dying off," and the latter character appeared to be inherited by a large proportion of them.

On becoming associated with Mr. Clark in Fiji in 1908, the writer soon formed the opinion that this "dying off" was due to a specific disease. Its incidence was too irregular to be accounted for by "early maturity," but corresponded closely with what will later be described as the acute form of leaf-scald. Furthermore, it was observed, particularly in one of the more resistant Mahona seedlings, that "dying off" was sometimes preceded by copious production of side-shoots with white etiolated leaves, these closely corresponding with advanced symptoms of the comparatively inconspicuous chronic form of leaf-scald.

Thus, strong presumptive evidence is furnished that leaf-scald was prevalent in Queensland and Fiji from about the year 1900 onwards. Whether it was introduced in 1896, along with the New Guinea canes, or at some earlier date is obscure. In view of its probable introduction from New Guinea, its future identification there is awaited with interest.

The earliest definite record of leaf-scald was made in 1911, when many sporadic examples of a strange disease were noted by the writer in the Herbert and Johnstone River districts of North Queensland. In 1915 the same disease was again observed in these two districts, and also in the Cairns and Mackay districts, severe losses having been caused by it that season in several varieties, notably Goru (N.G. 24). The symptoms noted were white etiolated leaves and copious production of side-shoots on dwarfed stalks, which slowly withered and died. No sudden dying off was heard of in 1911. Formalin specimens collected on the Johnstone River in 1915 have lately enabled definite identification of this disease as leaf-scald to be made, affected bundles containing the bacteria having been found in streaks on leaf-blades and leaf-sheaths and in stem tissues.

In 1915, the sudden dying off phase was also observed, particularly in Clark's Seedling (H.Q. 426), in the Mackay and Childers

districts. This was then, however, regarded as another distinct disease, and was described as a "wilt," although both phases were seen occurring together in Clark's Seedling at Mackay.

Its limitation to endemic proportions at this period may be accounted for by the previous disappearance of Mahona and other highly susceptible varieties from the districts concerned.

In New South Wales the disease was first noticed in 1912 in the Clarence River district. In a field of Mahona, at Garven's farm, a large proportion of the stools were dead, while the remainder, scattered amongst the dead ones, were mostly green and of healthy vigorous appearance. Odd stools of N.G. 16 and N.G. 14, similarly wilted, were also noted in other parts of the district.

At this time, Mahona was producing excellent crops, both one and two-year-old, in the Richmond and Tweed River districts, especially the former, where it was then the chief standard variety. These results prompted further trials on the Clarence, in spite of earlier failures. Blocks of Mahona were, therefore, planted on a number of farms in 1914. All these were later badly damaged by leaf-scald, some not being worth harvesting.

In September 1912, 46 tons of N.G. 16 seed cane were procured from the above-mentioned Garven's farm on the Clarence, for distribution throughout the Richmond River district. A "new" disease (actually leaf-scald) appeared in the resulting crops of this variety. It had caused considerable alarm by 1915, when the writer was sent to make a hurried preliminary investigation. It was found that, while the disease was prevalent in all the Mahona on the Clarence, it occurred in only a few blocks of this variety on the Tweed, suggesting that its introduction to the latter district had been recent. No affected Mahona was found on the Richmond, the disease being apparently still confined to the N.G. 16 introduced from the Clarence.

Thus, the position in 1915 was regarded as a critical one, particularly for the Richmond, where Mahona constituted 65 per cent. of the crop. It appeared that the introduction of this disease from the Clarence threatened not only severe losses, but the very existence of this highly favoured variety in the district. Recommendations were accordingly made for controlling it, based on its being transmissible by cuttings and infectious. These included avoidance for seed cane purposes of all fields showing the disease, and of all Mahona fields nearby such fields ; ploughing out after harvesting of badly diseased fields and eradication of diseased stools in other fields ; and discontinuance of Mahona planting on the Clarence.

Little was done to give effect to these recommendations until 1920, mainly owing to interruption of investigations by the war. In 1916 its appearance was reported in Mahona on the Richmond. In 1917, owing to further spreading, M. S. Barnett was sent to look into the position. He found it to be very general in both Mahona and N.G. 16 on the Richmond ; it was more localised on the Tweed, although certain fields of Mahona were seriously damaged by it ; and the few blocks of Mahona still remaining on the Clarence were in a bad way, although he found only odd stools affected of N.G. 16 and other varieties.

He repeated the writer's recommendations, suitably altered to meet later developments. But it proved impracticable to attempt serious control measures in the absence of competent technical advice on the spot.

It should also be noted that these observations in N.S.W. only concerned the conspicuous wilting phase. Its intimate relationship with the chronic phase was not recognised until late in 1919. This was a serious limitation, both to the completeness of the distribution recorded and to the efficacy of the measures recommended, particularly as symptoms of the chronic phase may manifest themselves in a new locality a year or two before the wilting phase appears.

Barnett also proposed the appropriate name of " leaf-scald," by which the disease has since become generally known. Previously the wilting phase had been variously referred to as " Mahona disease," " new disease," " withering disease," etc., in N.S.W., and as " dry rot " at the Mackay Government Experiment Station, where it was pointed out to me in 1915 by the manager at that time, Mr. McCready. The chronic phase was also known in Queensland by such names as " tip wither " and " white leaf."

Its present known distribution is as follows :—

New South Wales.—On the Clarence it has almost disappeared since Mahona was discarded. On the Richmond it is widespread in Mahona crops, but occurs rarely in N.G. 16 or other varieties. On the Tweed, all remaining Mahona crops are severely affected, and the variety is rapidly disappearing.

Queensland.—In the Cairns district it is widely distributed, Badila being slightly and Clark's Seedling severely affected. On the Herbert River it has become rare, although Clark's Seedling is widely grown. Observations are lacking for other districts, except that a case has lately been recorded in Badila on the Johnstone River.

Fiji.—An outbreak of moderate proportions occurred in 1923 at Rarawai Mill, mainly in variety 7R428. It also appeared at Nausori Mill in 1923 in some Mahona raised from cuttings sent from Rarawai in 1921. Previous to these outbreaks it was thought to have become extinct in Fiji.*

3. Losses and Varieties Affected.

It will already have been gathered that leaf-scald has chiefly been a disease of Mahona variety. The losses it causes have been studied in detail in this variety on the Richmond River, where it occupied an area of approximately 1,500 acres in each of the years 1920, 1921, 1922, and 1923.

Of this area, in 1920, 63% showed definite signs of infection. In 1921, the position had improved slightly to 52% infected, as a result of about 30% of the area being ploughed out after harvesting, and other areas of similar extent being replanted in 1920, mainly with specially selected healthy seed cane. In 1922, the area infected had again increased to 58%, in spite of similar control measures being again used. A further increase to 64% occurred in 1923. And as further rapid increase seems inevitable, planting of this variety is being discontinued.

An important factor in this failure to control the disease was complications due to gumming disease. It attacked Mahona crops in localities free from leaf-scald and reduced the quantity of "safe" seed cane available to such an extent that an increasing proportion of cane of doubtful soundness has been used for planting purposes. But, even so, the infection of new areas has been due less to the use of infected planting material than to spreading by secondary infection from field to field.

About 750 acres (half the total area) of Mahona were harvested each year. The losses were estimated in the diseased crops harvested and are summarised in the following table:—

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* Since the above was written (1923), various reports show it to be widely distributed in Queensland fairly generally, and to be causing severe damage in crops of susceptible varieties in a few localities. A great many Badila crops are affected, but this resistant variety rarely suffers severe damage. It appears to be particularly widespread in Badila, on the Johnstone River (Queensland) and Tweed River (N.S.W.), without causing appreciable losses in this variety.

In Fiji, the outbreak at Rarawai Mill in 7R428 has assumed more serious proportions, in spite of rigid control measures being exercised. In this variety, the symptoms are so fugitive that the selection of healthy planting material is rendered particularly difficult, in fact well-nigh impracticable. But it still has not been found in other districts, except at Nausori, as stated, where the prospects seem good that the measures adopted have again eradicated it.

It is now also considered to be identical with the gum disease of Java, as shown in the introduction, and with the similar disease in the Philippines, recorded by Lee (4 and 5).

Estimated Losses (Tons Cane) from Leaf-scald for Four Seasons According to Area (Acres) of Diseased Mahona Harvested.

Season.	Diseased Areas Harvested (Acres).			Total Losses Tons Cane.
	Severely Affected.	Slightly Affected.	Total.	
1920	65	360	425	940
1921	132	228	360	2,017
1922	235	223	458	2,179
1923	320	131	451	2,570
Total 4 Seasons	752	942	1,694	7,706

The bulk of these losses were, of course, attributed to the severely affected areas. Of these, eight fields, totalling 31 acres, were a total loss, not being worth harvesting, and alone accounted for an estimated loss of 535 tons of cane. On 378 acres losses ranged from 25% of the crop upwards, and on the remaining 343 acres from about 10% to 25%. The disease also caused considerable areas to be ploughed out after harvesting, instead of being ratooned.

The table includes no allowance for loss in quality in the diseased crops harvested, it being impracticable to determine this with any degree of accuracy. This loss is certainly considerable, but the decrease in quality varies greatly in different canes, according to the stage to which the disease has advanced; a proportion of diseased canes, which should properly be discarded as rotten and valueless, are inevitably sent to the mill.

It was similarly impracticable to compute the loss due to the fact that Mahona, which but for disease was the most highly favoured variety, has been to a large extent, and will be completely, discarded on account of the inroads of both leaf-scald and gumming diseases. Its place is being taken by other varieties, mainly Malabar, which, while more resistant to these diseases, are otherwise inferior to it in sugar-producing capacity.

In the Tweed River district, Mahona has suffered from leaf-scald more severely still. Late reports show the entire area of this variety to be now affected, a large proportion of it severely. And its planting is being discontinued on account of leaf-scald alone, the position there not being complicated by gumming or other disease. As Mahona has apparently met with a similar fate wherever it has been grown, the losses through having to discard this and other valuable varieties may conceivably outweigh all other losses combined.

The only other variety which has suffered considerably on the Richmond is N.G. 16. In 1920, 11 acres showed a loss of between

5% and 10% of the crop. No crop of this variety has been severely affected since then, but losses were more severe in earlier crops, particularly in 1919 and other dry seasons. Of these we have no detailed estimates.

The relative susceptibility of the varieties grown on a field scale on the Richmond is fairly accurately known, having been determined by systematic field observations extending over the past five years. But, as regards that of the varieties of other districts and of the numerous rarer varieties grown for trial purposes, our knowledge is fragmentary.

Our present ideas on this question are set forth in the following table, in which Mahona, N.G. 16, and Badila are taken as standards of high susceptibility, moderate resistance and high resistance respectively. Varieties about the susceptibility of which most is known are named first in order. No immune variety is known, occasional diseased stools having been encountered in every variety which has been observed constantly exposed to infection.

Relative Susceptibility of Cane Varieties to Leaf-scald.

Variety.	Districts in which Observed.	Susceptibility.	Remarks
Mahona	All districts.	Very susceptible.	
N.G. 16	Northern Rivers of N.S.W. only.	Moderately resistant.	
Badila	All districts.	Highly resistant.	Only moderately resistant in Nth. Queensland. esp. Cairns dist.
M. 1900 Sdg. ..	Northern Rivers of N S.W only.	Moderately resistant.	More resistant than N.G. 16, less than Badila.
Malabar ..			
D. 1135 ..	Northern Rivers	Highly	Similar to Badila
Innes 131 ..	of N.S.W. only.	resistant.	in N.S.W.
N.G. 14 ..			
Clark's Seedling (H.Q. 426) ..	Nth. Queensland & Richmond River.	Moderately resistant.	Highly susceptible in Cairns district. Similar to N.G. 16 in N.S.W.
Pompey (7 R. 428)	Rarawai and Richmond River.	Moderately resistant.	
Goru & Sports (N.G. 24, 24A, 24B)	Nth. Queensland.	Moderately resistant.	
8 R. 31	Richmond River & Rarawai.	Susceptible.	
7 R. 96	Richmond River & Tweed River.	Very susceptible.	

Variety.	Districts in which Observed.	Susceptibility.	Remarks.
H.Q. 243 (identity doubtful) ..	Tweed River.	Very susceptible.	
14 N.G. 146 ..	Rarawai.	Moderately resistant.	

Diseased stools have also been recently recorded in forty odd seedling varieties at Rarawai, Fiji.

4. Symptoms.

(*a*) External.—Two distinct phases occur, an acute form and a chronic form. The two phases are so different in appearance that they were at first regarded as belonging to two separate diseases. Either phase frequently occurs to the exclusion of the other. But more often they grade into each other, the one or the other predominating.

The Acute Form.—Affected plants suddenly wilt and die. The wilting takes place in the manner that occurs when a stool of cane is removed from the ground, or just as if it were suddenly deprived of the use of its whole root system. First the leaves wither. Then the stalk gradually shrivels and dries up. Commonly, whole stools are thus affected ; or it may be parts of stools, or only one or two stalks, when the remainder may retain a perfectly normal, vigorous appearance. On occasions whole fields are suddenly attacked in this manner, or larger or smaller patches in a field. But more often odd stools are singled out here and there. In the latter case, the withered diseased stools contrast strangely with the fine green appearance of the apparently unaffected stools alongside them, especially in well grown crops on good land.

The extreme rapidity of the wilting in certain cases is remarkable. A vigorous healthy looking stool may be transformed in a week or two into a withered object on which not a single green leaf remains. But such rapid wilting only occurs in the driest weather. Usually the process is considerably more prolonged.

On the three Northern Rivers of N.S.W. this phase occurs typically in one and two-year-old crops approaching maturity in the spring or early summer. It is most pronounced in the driest seasons, particularly after a month or two of continuous dry weather. On the other hand, in seasons of plentiful, evenly-distributed rainfall, this phase may be practically absent. On this account farmers early came to regard the disease as one of intermittent seasonal occurrence, the chronic form being of comparatively inconspicuous appearance and unnoticed by them.

Sometimes wilting also continues throughout the summer, autumn and winter, in crops over one-year-old. But it is usually then a more gradual process and more definitely associated with the chronic form, although leaf-streaks are frequently rare and difficult to locate.

Stools affected solely by the acute form may show no characteristic symptoms by which they can be definitely distinguished from ordinary dead or dying stools (*e.g.*, those killed by the Queensland cane grubs (*Lepidoderma albohirtum*, Waterh.)). Careful search at the base of the wilted stools, however, will frequently reveal one or more tiny diseased shoots springing either from the ground or from the side of a stalk and displaying the critical leaf symptoms of the chronic form. The type of shoot is shown in Plate 2 (B and C), and in figures 4 and 8. This enables a definite diagnosis as leaf-scald to be made. Failing such shoots, diagnosis can only be made after harvesting and ratooning the affected stools, when usually sufficient new shoots spring up for accurate diagnostic purposes. If, however, the disease has progressed too far before ratooning, the stool will be completely dead, and no means of diagnosis will remain. It also happens, more especially in the case of N.G. 16 and others of the more resistant varieties, that on ratooning an apparently healthy stool is at first produced and that the critical symptoms do not appear in it for some time, perhaps not until the following spring, when it is about a year old.

The Chronic Form.—The one critical symptom externally visible which enables a positive diagnosis to be made, consists of the whitish streaks on the leaf-blades or leaf-sheaths, as shown in Plates 1 and 2 and in Figs. 7, 8, 9, 10 and 13. They are straight, narrow, well-defined streaks, ranging from $\frac{1}{8}$" in width down to a barely perceptible mark, and often extend throughout the entire length of the leaf-blade and leaf-sheath (running out on the margin towards the leaf-tip). Some of them, especially the narrower ones, however, become imperceptible after travelling a certain length, a feature which is best shown in Plate 1, A. The remarkable straight-

Explanation of Plate 1 (opposite).—Leaves of diseased Mahona (one-year-old ratoons), showing characteristic leaf-streaks. A is the lower half of an older leaf (the 6th from the apical spindle), while B and C are the lower and upper halves respectively of a younger leaf (the 3rd from the apical spindle).

In B and C, the streak on the midrib commences in the leaf-sheath, and runs the full length of the leaf-blade, terminating as a broad withered wedge at the leaf-tip. The streak to the left is similar, running out in the withered portion on the leaf margin. The tendency of the streaks to become broad and diffused as a preliminary to withering may be observed in C.

In A, the streak to the right is still for the most part narrow and well defined, but that to the left has become broadened and diffused, except towards the base of the leaf-blade. An unusually large, broad streak appears on the midrib, containing pronounced red blotches. Just to the right of the midrib there is a narrow streak, which fades away after running only about one-sixth of the length of the leaf-blade. Several still smaller streaks may also be seen some of which are confined to the leaf-sheath.

Drawn end of October, 1921. ×3/5.

LEAF SCALD DISEASE

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ness and evenness of the streaks depends on the fact that they strictly follow the course of one or a group of several vascular bundles throughout their entire length. Their colour ranges from almost pure white to cream or pale grey. The streaks become

FIG. 1.—One-year-plant Mahona, with a typical diseased stalk (chronic phase) in the centre foreground, from which the trash has been removed. It is considerably stunted, having short internodes and 12 side-shoots. Its younger leaves are severely etiolated (white), shortened, have withered tips, and show a marked tendency to curl inwards, bow shaped. Photographed 29/8/19.

plainly visible as soon as the young leaf unfolds and turns green. Some may even be detected by close examination, in the white immature leaves unfolded by hand from the apical spindle of unopened leaves.

As the leaves grow older the streaks usually tend to broaden and become more diffused, losing their sharply defined even margins. Sometimes irregular reddening of the tissues also occurs, especially in the centre of the streak. This broadening is later followed by withering of the leaf tissues involved. As the process of broadening and withering usually commences at the tip of the leaf and works downwards, typical streaks are narrow and well defined at the base of the leaf-blade, becoming more diffused higher up and ending in a broad withered portion towards the leaf tip. Some streaks, however, become diffused or withered throughout their entire length, and others only in a central portion, the part higher up remaining well defined. The broadening and withering process does not develop in conformity with the age of the leaves, as streaks in different stages are frequently found on one and the same leaf (Plate 1, A). It develops more rapidly in dry than in wet weather, in severely than in slightly affected plants, and so forth. In young vigorous crops, up to, say, six-months-old, narrow white well-defined streaks without withering are the rule. Whereas, in one-year-old cane and older they are only seen to advantage in good wet spring and early summer seasons, when the cane is growing vigorously. In dry spring and later seasons they may be visible only on the leaf-sheaths, or they are often completely obscured by withering, so far as the leaves of the main stem are concerned.

Another prominent feature of the chronic form is the production of side-shoots and of small suckers (Plate 2 and Figs. 1, 2, 3, 4, 5, 6, 7, and 8). The tendency to shoot in this manner is even more pronounced than in the case of gumming or Fiji disease. For it is usual for the buds to sprout at every single node, both below and above the ground, even those of the immature tissues of the cane-top, almost to the growing point (Plate 2, A). These shoots have a

Explanation of Plate 2 (opposite).—Portions of diseased Mahona, one-year-old ratoons

A. Top of stalk split longitudinally, displaying the reddened vascular bundles, chiefly in the nodes, but also pronounced in the internodes. At two spots, the parenchyma has been invaded, with a tendency to form cavities. Reddened bundles may be seen in the embryonic tissues within quarter-inch of the growing point and in the lateral buds. They are also numerous in the leaf-sheaths. All the lateral buds are shooting, even the youngest ones.

B. Two nodes from the middle portion of stalk, with side shoots attached. Leaf-streaks are plainly visible on the left-hand shoot, especially on the leaf-sheaths. They have been obscured by etiolation and discolouration on the leaf-sheaths of the right-hand shoot, of which the leaf-blades (which were removed) were all withered.

C. A small side-shoot from the lower portion of a less severely diseased stalk. A well-defined white leaf-streak is visible on one leaf-blade.

Drawn November 1st, 1921. ×1/1.

LEAF SCALD DISEASE

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miserable, attenuated appearance. The symptoms they display are exactly analagous to those of the tops of the main stems. Their great value for diagnostic purposes lies in the fact that frequently the leaf-streaks may be found on them, when they have been effectively obscured by withering on all other parts of the stool

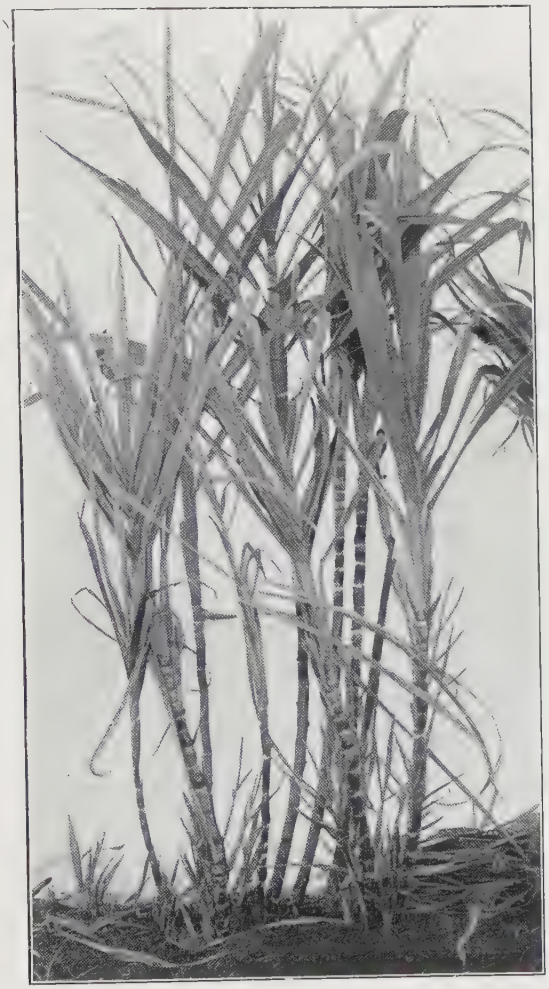


FIG. 2.—Two diseased stools of one-year-ratoon Mahona. The three stalks in front with side-shoots show symptoms of the chronic phase, being dwarfed and having the usual leaf-symptoms, but not very advanced. The two small stalks have dead tops, that of one being shrivelled up, and a few side-shoots. The four tall stalks behind are of normal healthy appearance. Numerous small suckers have also sprung from the ground; they are more or less withered, and closely resemble the side-shoots in appearance. Photographed 13/12/21.

concerned. They do not long survive, however, usually dying after attaining a length (including leaves) of from 6 to 18 inches. Those shown in Fig. 6 are unusually large and free from withering.

In one-year-old cane, sometimes the sprouting of one or two side-shoots is the very first symptom to appear, although usually

FIG. 3.—Another stool of one-year-ratoon Mahona, more advanced stage of disease than Fig. 2. One stalk only of normal healthy appearance remains. Photographed 13/12/21.

these and the leaf-streaks appear more or less simultaneously. Accurate determination of the symptoms was first undertaken during August, September and October, 1919. The method fol-

lowed was to tie a label on each of a number of selected stalks, which were examined about once a week. At each examination the symptoms of each stalk and their development were noted on the labels. Amongst these were labelled stalks of healthy appearance in every respect, except that one, two or three small side-shoots had sprouted from alternate nodes all on one side of the stalk. In each case five or six side-shoots formed on the one side before any shot on the opposite side of the stalk. The first shoots to form were later found to be the first to die, all of the buds on both sides eventually

FIG. 4.—Base of the stool shown in Fig. 3, showing the diseased appearance of the small suckers . Many of their leaves are withered, either wholly or in part, some being shrivelled, and they exhibit leaf-streaks and the other usual symptoms of the disease. Photographed 14/12/21.

sprouting and leaf-streaks and other symptoms developing. This peculiar phenomenon, which was found later to occur frequently, will again be referred to.

Pronounced etiolation of the leaves also occurs in the more advanced stages of the disease in canes of all ages. Etiolated leaves, mainly white, are shown in Figs. 1, 6, 8, 10, 11, 12, 13 and in Plate 2, B, on the two large side-shoots. Usually the young leaves of a stalk display this feature, as premature withering obscures it as the leaves get older. The white colour at first effectively masks any

leaf-streaks that may be present, but the latter show up by withering earlier than the rest of the leaf (Fig. 13). While not to be relied on alone for diagnostic purposes, the white leaves stand out clearly from a distance, and serve to indicate plants in which the leaf-streaks may be looked for and a definite diagnosis obtained.

FIG. 5.—Upper portions of a diseased and an apparently healthy stalk of one-year-ratoon Mahona from adjoining stools. Here again the characteristic leaf symptoms are seen, both in the cane-top and in the side-shoots. Photographed 13/12/21.

Another feature which serves the same purpose is the tendency of affected leaves to curl upwards and inwards in the form of a bow (Figs. 1, 2, 3, 5, 6 7, 8, 11). It is always associated with the white

FIG. 6. –Upper portion of a diseased stalk of one-year-plant Mahona, taken in the early spring. Compare with Fig. 5, taken in the summer. The side-shoots, although unusually large, are still green, and devoid of pronounced symptoms other than leaf-streaks. The cane-top is of compact shape, as the growing-point is not pushing out with the renewed spring growth. Of the numerous leaf-streaks present, only one is plainly visible, on the leaf-sheath to the left. Stunted leaves, etiolation, withered leaf-tips and inward curling are pronounced. Photographed 29/8/19.

etiolated leaves, but is often present without them. In combination with the withered leaf-tips, it gives to the diseased stalks a highly characteristic appearance, which at once attracts the attention of one familiar with it.

(*b*) INTERNAL.—When a diseased stalk not too severely af-

FIG. 7.—Upper portion of a diseased stalk of one-year-ratoon Mahona, less advanced stage than Figs. 5 and 6. Leaves display withered tips, inward curling, white and withered leaf-streaks. The side-shoots are still small and green. Photographed 14/12/21.

fected, such as that shown in Fig. 7, is split and viewed in longitudinal section, more or less discolouration will be seen throughout its entire length. The nature of this discolouration is shown in Plate 2, A, which represents the top of such a stalk, the appearance in all parts of the stalk being much the same. It consists of tiny

bright red streaks, which, on closer examination, are found to be reddened fibres (vascular bundles). Many of the red streaks are very minute, not nearly as thick as the vascular bundle containing them. Most are seen in the nodes, where the bundles run in all directions. But some also extend into or even right through the internodes. They can be seen most plainly in the mature tissues,

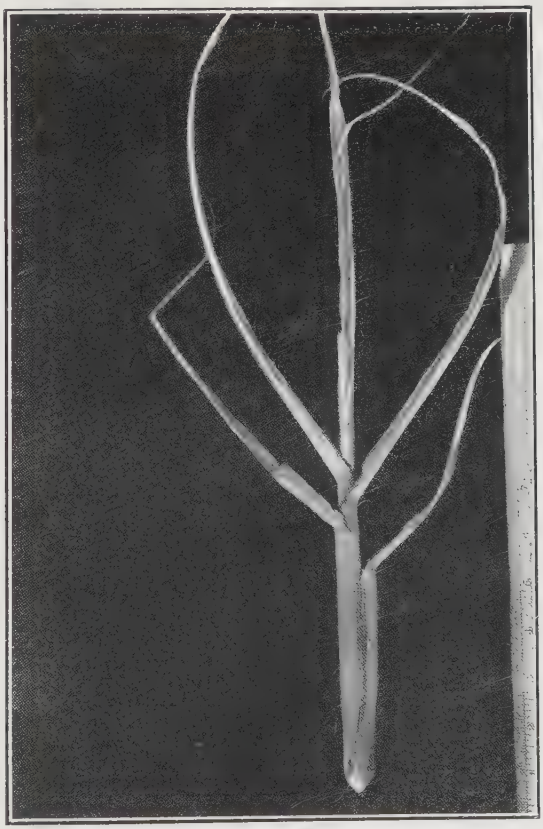


FIG. 8.—A large side-shoot of one-year-ratoon Mahona. The greater part of each leaf-blade is withered and shrivelled, except the youngest leaves which are white (etiolated). Leaf-streaks are distinguishable on the two left-hand leaf-blades, and running down the leaf-sheaths. Photographed 16/12/21.

but they are also visible in the immature tissues close to the growing point, and can nearly always be distinguished in the embryonic stem tissues of the side-shoots, even of those which are only just commencing to sprout (Plate 2, A). It is astonishing to find such marked derangement of the vital tissues near the growing-point accompanied by so little outwardly visible damage to the cane-top.

FIG. 9.—Lower half of leaf-blade, 6th leaf from apical spindle, displaying leaf-streaks. A withered streak may be seen near the left-hand edge. The streak near the midrib to the right is also withered towards the top. That near the midrib to the left becomes invisible towards the top. The largest streak is on the midrib and runs the full length. All are traceable into the leaf-sheath. Photographed 16/12/21. × 1/2.

Many stalks are fairly evenly marked in this manner throughout their length, but others are more reddened at one part than another. The most reddened nodes bear either the largest side-shoots or the shoots with the most severe symptoms. Reddening is rarely found in nodes from which side-shoots have not sprouted. Similarly, tops of stalks with much reddening in the tissues show many leaf-streaks and much etiolation of the leaves.

There is, however, no such association of reddened tissues with withering. This is seen especially on dissecting stalks dying of the acute form of the disease. Provided that they have produced no side-shoots, their internal tissues appear perfectly normal in every respect, or if they be examined long after wilting, their tissues will merely be more or less dry and shrivelled, like those of stale healthy cut stalks. If, however, as frequently occurs, these stalks have produced a diseased side-shoot or two at their base (either below or above ground), their tissues at that point will be reddened accordingly.

Similar reddening of the vascular bundles occurs also in the streaks on leaf-sheaths (Plate 2, A) and leaf-blades (Plate 1). The fact is that both the leaf-streaks and the reddening serve to indicate the vascular bundles which have been invaded by the bacteria, although all the invaded bundles are not reddened. Thus, we have pure white leaf-streaks, both superficially and in section, superficially white leaf-streaks which show red bundles in section, and red bundles in leaves of which no indication can be detected superficially. The latter may be regarded as deep-seated leaf-streaks hidden from

view by the outer layers of leaf-tissue. We similarly sometimes find leaf-streaks visible only on one surface of the leaf, either the upper or the lower surface. These deep hidden streaks are common in the leaf-sheaths.

In the more advanced stages of the disease other tissues besides the bundles may be invaded at certain spots, smaller or larger dried up cavities being ultimately formed. The beginnings of two such cavities in the stem are shown in Plate 2, A. While part of the tissues surrounding the cavities may be light or dark red, sometimes nearly black, other tissues become opaque white and dry looking. Greyish brown necrotic tissues sometimes also occur.

The reddened bundles, especially those in the stem, are useful for diagnostic purposes, particularly as a means of confirmation in

FIG. 10.—Diseased Mahona plants three-months-old, resulting from the inoculation of healthy cuttings with a pure culture of the organism. They were inoculated by dropping ½ cc. of the culture fluid on to each cut end, and planted on 29/9/21. (Inoculation test 3.) The photograph was taken on 24/12/21.

Plant B germinated first, shoot 1 having appeared one month after planting, and shoot 2 another month later. But growth soon came to a standstill in both, and they died out when four-months-old.

In box A the main shoot 3 appeared about five weeks after planting and then grew vigorously, in a normal healthy manner. The first symptom of disease, a fine white leaf-streak on a newly unfolded leaf was found on 19/12/21. This is clearly displayed on the upper left-hand large leaf in the photograph.

doubtful cases. In wilted canes it is sometimes possible to detect them at the butts of stalks when no leaf-streaks or other means of positive determination can be found (*e.g.*, when side-shoots, formed at the base, have died). But as they cannot be distinguished by the naked eye, from similar reddened bundles due to many other causes, microscopic examination of them becomes necessary if definite diagnosis be desired. Similarly, in very early stages stalks which, in all other respects, appear healthy, may be suspected as a result of one or more side-shoots commencing to sprout. A reddened bundle or two may be detected in the node to which the shoot is attached, and definite diagnosis as leaf-scald may again be made by finding the bacteria microscopically in it.

(*c*) Roots.—Diseased stools have been dug up and their roots

A B

Fig. 11.—Two further Mahona plants from Inoculation Test 3, for comparison with those shown in Fig. 10 and with each other. The diseased plant (A) resulted from an inoculated cutting, the other (B) being a healthy control plant. Both were planted on 29/9/21 and photographed 22/12/21.

The two plants germinated at the same time, each producing two shoots one month after planting. The control plant grew in a normal healthy manner throughout. But both shoots of the inoculated plant had white etiolated leaves at first, although some more nearly normal leaves appeared later. Note the withered leaf-tips and the stiff, inward curling leaves.

The three diseased plants shown in these two photographs serve well to illustrate the appearance of diseased plants in fields of young cane. Sickly shoots with white leaves, like those shown, are common in the field, but strong shoots of normal appearance, except for a well-defined leaf-streak or two (of the kind shown in Fig. 10), are still more common.

carefully examined and compared with those of healthy stools. But so far no abnormality in roots characteristic of leaf-scald has been discovered, except in the case where diseased cuttings have been planted (see p. 69, "Cuttings from stalks affected by the chronic phase"). With these, root development is largely suppressed, and the few weak roots which sprout soon wither.

It is, however, considered that far too little investigation of the roots has yet been made, as will later (p 62.) be explained more fully.

FIG. 12. 1 2

FIG. 12.—Closer view of the inoculated plant shown in Fig. 11. In shoot 2 all the leaves are almost white. This shoot died 6 weeks later. In shoot 1 the four youngest green leaves are of healthy appearance, showing neither a leaf-streak nor any other symptom. In the 5th leaf, etiolation shows in the form of broad white stripes. The 6th leaf is white below, but withered above. This shoot produced a further series of apparently healthy leaves. It was not until 9/2/22 (6 weeks later) that a leaf unfolded bearing a characteristic leaf-streak, after which, however, the disease developed rapidly.

5. Aetiology.

(*a*) The Causal Organism in the Tissues.—The readiest means of finding the bacteria is by preparing stained slides from

Fig. 13.—Pieces of etiolated leaves from 3-months-plant Mahona. A and B are the lower and upper portions of the same leaf-blade. A leaf-streak which runs to the left of the midrib is distinguishable only in the upper portion, where it has withered. Lower down it is more or less masked by the etiolation. The portion of leaf shown in C similarly contains two leaf-streaks scarcely distinguishable except in the upper withered portions of the streaks. Photographed 16/12/21.

small portions of leaf-streaks, or of stem tissues containing discoloured bundles, well crushed up with water. In Fig. 14, the bacteria of gumming and of leaf-scald, as seen in slides of this kind, are compared. In the cane-tissues the leaf-scald bacteria are smaller than those of gumming, particularly more slender. They also stain less readily. The difference is sufficiently marked to provide a convenient and sure means of distinguishing between the two diseases.

The bacteria proved difficult to demonstrate in sections, being both unusually small and difficult to stain. Some motile individuals may be seen in sections of fresh material without staining. But stained free-hand sections, prepared by methods which succeeded well with gumming, gave no satisfaction.

Serial paraffin sections gave the best results. The material was fixed in alcohol (95% or 98%), or in Bouin's Picro-formol (the latter proving slightly preferable). The sections were stained for long periods (over-night to 48 hours) in undiluted Ziehl's Carbol-fuchsin, differentiated and dehydrated in a graduated series of alcohols, cleared in xylol and mounted in balsam.

FIG. 14. A.—*Bacterium vascularum* (Cobb) Greig-Smith (for comparison with B). Smear of gum oozed from cut end of cutting of D.1135, inoculated with a pure culture one month previously. Stained dilute carbol fuchsin 20/3/22.

B.—Leaf-scald bacteria from white leaf-streak in Mahona, inoculated with a pure culture two months previously. Material crushed on slide and stained dil. carbol fuchsin 20/3/22.

(NOTE.—The debris of the plant tissues are not shown, nor do the bacteria belong to a single group as seen on the slide. Comparison with Fig. 20, B, suggests that the larger forms are shown in too large a proportion, to the exclusion of the smaller forms.)

Figs. 15-19 are camera lucida drawings from sections prepared in this manner. Figs. 15, 16, and 17 are all of the same streak. It was a large pronounced streak on a leaf-sheath of a small shoot of Mahona, two-months-old ratoons, collected 7/2/22. All the unfolded leaves of the shoot were of normal healthy appearance, except for this one leaf (the third from the central spindle), which contained the single white streak running through both leaf-blade and leaf-sheath. The leaf-sheath was green just below its

junction with the leaf-blade but white and immature lower down. The streak had split open for several inches on the inner surface of the leaf-sheath, displaying broken down, more or less reddened

FIG. 15.—Transverse section of white immature lower portion of a leaf-sheath of Mahona from a small shoot. A large pronounced leaf-streak is located between the two small sized vascular bundles, its limits being roughly indicated by the shrunken parenchyma cells.

The tissues of the large vascular bundle in the centre of the streak have been nearly all broken down and replaced by two bacterial cavities. The walls of the cavities are lined with bacteria and cell debris, but apparently most of the contents has been discharged through the longitudinal split on the inner surface of the leaf-sheath. This bundle was one of similar size and type to the large one on the extreme left of normal appearance.

The smaller cavity enclosed in a circle is shown in Fig. 16, more highly magnified.

tissues within, while both above and below the split it was mainly white with slight irregular reddening here and there.

The split had evidently resulted from the formation of a large bacterial cavity in the streak, a large vascular bundle as well as some of the surrounding tissues, being completely broken down.

Fig. 15 was drawn from a section from the lower end of the split part of the streak, where the tissues were immature and soft. The cavity is here divided into two parts. Its walls are lined with bacteria and cell debris, as may be seen in Fig. 16, which is a detail from Fig. 15, showing the portion surrounded by a circle more highly magnified. Most of the contents of the cavities had apparently been discharged through the split. The broken down

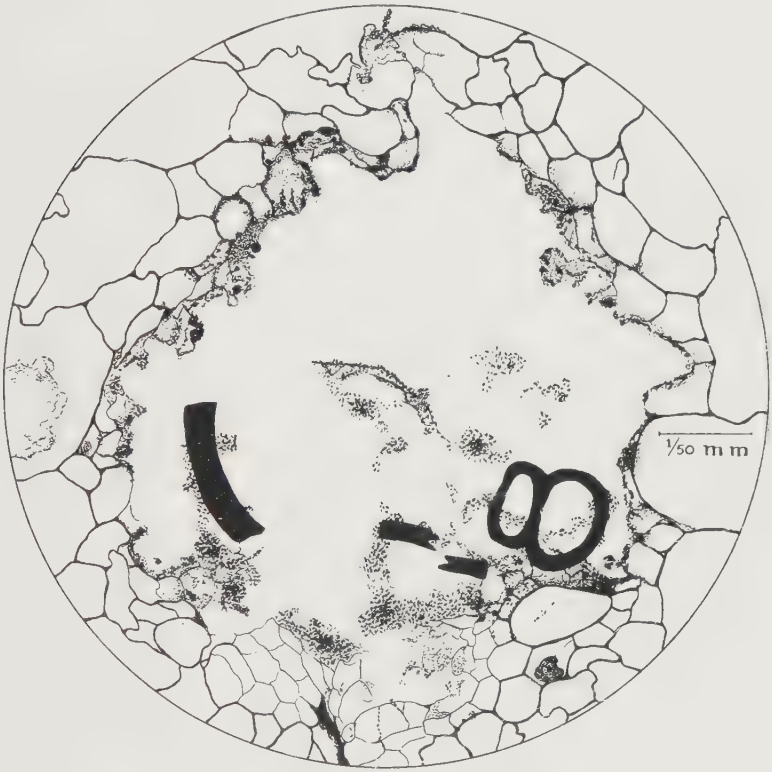


Fig. 16.—A detail from Fig. 15, showing the bacteria mixed with cell debris in the smaller cavity. The surrounding parenchyma cells were not apparently invaded except on the immediate margins of the cavity.

vascular bundle should have been of similar size and type to the normal one shown on the extreme left in Fig. 15.

The same affected bundle is again shown in Fig. 17, but in this case in a section of the green upper portion of the leaf-sheath well above the split. Here the cavity is smaller, the bundle sheath being still intact. Much of the xylem has disappeared, however, and one of the remaining large pitted vessels is blocked with bacteria. The phloem is also partly broken down and strongly invaded by bacteria.

The above is a typical case of a leaf-sheath streak splitting open, with consequent discharge of large masses of bacteria to the exterior. As this phenomenon is of frequent occurrence in the case

FIG. 17. Transverse section of the same vascular bundle seen in Figs. 15 and 16, but 5 inches higher up in the leaf-sheath, where it was green and mature, being only one inch below its junction with the leaf-blade. This was well above the split portion of the streak. Here the cavity is much smaller, comprising little more than the lysigenous space of the bundle. The remains of the xylem parenchyma cells appear as if folded up on the walls of the cavity, in which few bacteria can be seen. Bacteria are numerous, however, just above and in the phloem tissues which are in process of disintegration. The left-hand large pitted vessel is blocked by a mass of bacteria embedded in an unstained gelatinous matrix, which keeps them separated and renders them clearly visible.

of leaf-sheath streaks, and has also been noted in rare cases with leaf-blade streaks, the question arises whether it is in any way connected with the dissemination of the disease. So far no answer to this question can be suggested.

In bundles, in which no breaking down of the tissues has taken place, the bacteria are usually confined strictly to the xylem elements. A typical appearance is to find one or more of the small pitted or spiral vessels blocked with a dense mass of bacteria, with few or none visible in the remaining vessels. Occasionally a large pitted vessel, or the annular vessel and air space may be found similarly blocked with a large bacterial mass. But in these large vessels the bacteria are more usually scattered or adhering to the walls in groups. Fig. 18 shows a pitted vessel of small size containing one of these dense masses of bacteria. And Fig. 19 shows the walls of a still smaller pitted vessel, at a part where the bacteria are thinly distributed, and illustrates a characteristic arrangement of the bacteria enmeshed in the pits of the wall.

Fig. 18.—Longitudinal section of a fine narrow, white leaf-streak in the youngest green leaf of a young shoot of Mahona. collected 7/2/22, showing part of one of the smallest-sized vascular bundles.

A mass of bacteria blocks up the pitted vessel in the centre of the drawing. No bacteria were distinguishable either in the phloem tissues on the right or in the bundle-sheath cells to the left.

In the paraffin sections, stained regressively as described, the bacteria appear smaller in size than they do when fixed by heat and stained progressively. The relative sizes may be seen in Fig. 20, B showing bacteria crushed from cane tissues and stained on the slide, and C and D bacteria in two different sections.

Some of the vessels, especially the larger ones, in the affected bundles are also blocked with a resin-like substance, of the kind described by Erwin F. Smith (7, p. 16) as "red pigment," "red stain," etc., and by Krüger and others in Java as "resin" or "wound-gum" in sereh. A similar substance, of course, occurs with many diseases. With leaf-scald, in unstained sections it ranges from a transparent pale brown or yellow to a bright orange red. In sections stained with fuchsin, it varies from transparent, almost colourless, to a dense red, almost black.

Bacteria, scattered either thinly or thickly, may be embedded in it; but more usually no bacteria can be detected. Masses of this substance and of bacteria may alternate with each other in the one vessel, the one merging into the other, either abruptly or gradually. When the substance is

transparent, stained bacteria embedded in it stand out clearly, as shown in the large pitted vessel in Fig. 17. But when it is dark, any bacteria which may be present are obscured.

That leaf-streaks may be visible on only one surface, either the upper or the lower, or not visible on either surface as already described (p. 28), is readily explained by the fact that the attack of the bacteria may be centred in a differently placed vessel or vessels in different vascular bundles. Thus invasion of the smallest sized bundle of the leaf-blade may show as a streak only on the lower surface; of a single small vessel high up in a large bundle, as a streak only on the upper surface; of a small vessel in the middle region, on neither surface; and so on. The ordinary pronounced streaks which show plainly on both surfaces are due to more general invasion of a larger vascular bundle, with sometimes cavity formation in addition; or to similar invasion of two or more adjoining bundles.

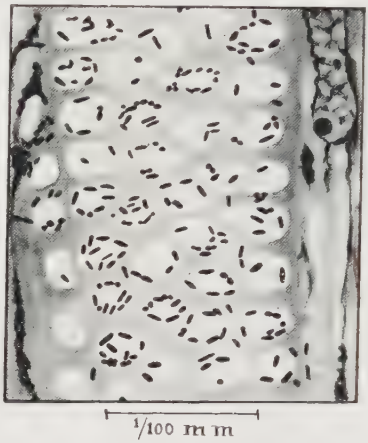


FIG. 19.—Longitudinal section of a narrow, white streak in a leaf-blade (another section of the same material, as shown in Fig. 18), showing portion of the pitted cell-wall of a small vessel, with bacteria enmeshed in the pits.

The arrangement of the bacteria shown has been frequently observed in those parts of pitted vessels in which only a few bacteria are present.

In narrow, sharply-defined leaf-streaks the bacteria are usually confined to the single bundle, or to the two or more bundles concerned. But as the streaks of mature leaves broaden and become more diffused preparatory to withering, as already described, the bacteria appear successively in the new bundles involved. This lateral spreading is believed to occur by way of the minute lateral connecting bundles, because bacteria, frequently in dense masses, have been seen in these in transverse sections of leaves.

In these cases, the originally invaded bundle is distinguishable by the absence of chloroplasts from the bundle-sheath and surrounding parenchyma. Around the newly involved bundles the chloroplasts do not similarly disappear, although they become more or less reduced in size and disorganised.

(*b*) Artificial Cultures.—Much difficulty was at first experienced in isolating and cultivating the organism in a satisfactory manner. Repeated trials were made in such media as nutrient agar, glucose agar, invert-sugar agar, cane-juice peptone agar, sucrose potato agar, sterilised pared pieces of cane, cane leaves, and a similar range of fluid media. Growth was ultimately obtained in most of them, but only in rare cases and after heavy seeding with the organism. Sucrose potato agar proved preferable to the other solid media, while sterile water served almost as well as any of the fluid media. But none served for the preparation of undoubtedly pure cultures, because the colonies which grew did not appear to bear a definite relation to the number of organisms used for seeding. Not one could even be relied upon to grow it without fail, after heavy seeding.

Further, the organism in these cultures differed widely in appearance from the normal straight slender rods found in the tissues, appearing in a great variety of fantastic involution forms which will be described later. All the circumstances suggested unfavourable conditions of growth resulting in abnormal cultures, lacking vigour, and therefore unsuitable for inoculation tests and for studying the relations of host and parasite generally. For these purposes it was most important that means should be found whereby the organism could readily be (1) isolated from the sugar cane tissues containing it, (2) grown in undoubtedly pure culture, by obtaining isolated colonies arising from single cells, and (3) grown and maintained in cultures in a normal, vigorous condition.

After an extensive series of cultures, in which various temperatures for incubation, media of varying reactions, and media varied in many other respects were tried, it was ultimately found that sufficiently vigorous growth to meet all these requirements could be obtained by the addition of blood serum to certain agar media.

Uncooked Albumen a Requirement.—On adding pieces of a diseased stem or of a leaf-streak aseptically to test tubes containing a little sterile water, usually a faint cloud develops in the fluid. The cloud may appear as soon as three days in rare cases, but more often it takes 6 to 10 days, sometimes as long as 20 days, or it may not develop at all. Various culture fluids used in place of the water gave similar results. The cloud results from growth of the organism in pure culture, provided that fresh young green material has been used and contamination avoided.

Efforts to subculture the organism from these cultures met with little success in media without blood serum. It failed to grow with sterilised pared pieces of cane or cane leaves standing in water, and in pure (boiled and filtered) cane juice. But it grew with raw pieces of cane (with water) and in raw cane juice, obtained sterile by tubing aseptically directly from healthy

cane. It usually failed to grow also on the agar media. Patchy growth on the agar surface was sometimes obtainable by heavy seeding, especially with sucrose potato agar. But growth was often confined to the condensed water, from which it might or might not be later transferable to the agar surface above by re-smearing. But it usually grows fairly readily on blood-serum sucrose potato agar, even when lightly seeded and colonies arising from single cells are freely produced. In fluid media the improvement by adding blood serum was much less pronounced, but growth in lightly seeded tubes occurred more often with than without it. Löfflers inspissated blood serum, and a similar medium, in which sucrose potato juice was substituted for the bouillon, presumably owing to being heated during preparation, proved just as poor as the various agar media without serum.

Thus it appeared that heating (presumably by coagulating albuminous substances) rendered cane tissues, cane juice and blood serum unsuitable for the organism, but that this deficiency could be restored, at any rate in part, by the addition of unheated blood serum. Possibly other substances, such as egg albumen, would also serve, but they have not yet been tried.

Serum from bullocks' blood was used. Of this, 10% was added to the various media. Smaller quantities gave proportionately poorer growth, but the addition of double that quantity made no noticeable difference. The serum needs to be mixed with the agar while molten; smearing a small quantity over the agar surface was not found to be advantageous. The blood serum used may be sterilised with ether, which is driven off at 55° C. before use, without noticeably affecting the growth of the organism.

Media Composed of Sugar Cane.—It is remarkable that so many of the media derived from cane which were tried proved unsuitable for the organism, even with the addition of blood serum. Most of those tried were prepared from the susceptible variety, Mahona, and included sterilised pared pieces from both mature and immature portions of the stem, mature and immature leaves, juice from mature and unripe stalks and from cane-tops, and a watery infusion of cane leaves. The juices were tried both as fluids and in agar media. In all of them, the organism usually failed to grow. At best, feeble growth occurred and in rare cases only.

But marked improvement resulted from diluting cane juice and adding peptone. 25%-cane-juice peptone water (consisting of cane-juice 25, water 75, peptone 0.5) proved a useful fluid medium, and was widely used, both with and without the addition of blood serum. Good growth was also obtained in some cases by heavy seeding on 25%-cane-juice peptone agar (the same with 2% agar added) and this medium with the addition of blood serum, proved one of the best of all, second only to blood-serum sucrose potato agar. On the other hand, 50%-cane-juice peptone water and agar (cane-juice 50, water 50, peptone 0.5), which are splendid media for *Bact. vascularum*, proved of little use, yielding fair growth in rare cases only, either with or without blood serum.

Juice from either mature or unripe stalks served equally well for preparing these media, but juice from cane-tops led to inferior growth of the organism. And infusions of cane leaves in similar combinations proved useless. In preparing the media, the cane juice was added after dissolving the agar and peptone, but prior to filtering. Potassium, sodium and calcium hydrate were each tried for adjustment of reaction, but which was used apparently made little difference.

Potato Juice Media.—As already indicated the best media tried were those derived from potato juice. It requires neither the addition of nitrogenous substances nor adjustment of reaction to render it suitable for the organism. Somewhat better growth, however, resulted from the addition of certain carbohydrates, especially sucrose. The great advantage of additions also of blood-serum have already been mentioned.

To prepare the juice, cleaned pared potatoes, in water, were quickly grated on a perforated tin grater, and the juice expressed with a meat press. The starch was got rid of by allowing several hours subsidence in narrow-necked stoppered bottles filled to the neck (to avoid oxidation), and the juice siphoned off, boiled and filtered. A clear pale amber-coloured fluid resulted. This stock juice was used in 25% dilution in the various media, the chief ones used being sucrose potato juice (sucrose 2, potato juice 25, water 75), and sucrose potato agar (the same with 2% agar).

The organism grows fairly well when sucrose is omitted from these media. But with the object of arriving at the most favourable medium obtainable, potato agar was tried with the addition of 1% of various carbohydrates. None appeared better than sucrose. Dextrose and laevulose appeared quite as good, or almost so. Mixtures of these (invert sugar, sucrose and glucose, sucrose and invert sugar) showed no decided improvement. Maltose, lactose, galactose, mannite, dextrine and starch seemed inferior. The latter all gave little, if any, better growth than the potato agar without any addition. Several of them, however, were obviously not inert, as they exerted a considerable influence on the form in which the organism grew. It will, therefore, be of interest later to determine the carbohydrate reactions of the organism. But a suitable carbohydrate-free medium for this purpose was not devised.

Other Vegetable Extract Media.—Media were also tried, prepared from swede turnips, carrots, prunes, oatmeal and haricot beans, each being used in a variety of combinations.

The juice from swede turnips, prepared in the same manner as potato juice, served nearly as well. But as no marked differences in the behaviour of the organism could be noted in the various combinations tried, the turnip juice appeared to offer no special advantages over potato juice.

None of the other materials yielded results of any promise.

Further investigations in this direction seem desirable, especially as the materials named were chosen more for their ready availability, than from consideration of their composition.

Beef Extract Media.—On nutrient agar, the organism usually fails to grow. Fair growth in patches sometimes follows heavy seeding, but attempts failed to maintain it through more than two or three subcultures on this medium. Even the addition of blood serum to it does not make much improvement.

But the addition of glucose (1%) provided a much more favourable medium. Vigour of growth on glucose agar is only slightly inferior to that on sucrose potato agar. But growth is more patchy and failures to grow more frequent.

Glucose being for a short period unobtainable, some invert sugar was prepared as a substitute, by heating sucrose (20 gms.) with hydrochloric acid (½ cc.) in water (to 100 cc.) at 70° C. for 60 minutes, sodium hydrate being added to slight alkalinity before sterilisation. While giving similar results

in most respects, the organism seemed to grow rather better on this "invert-sugar" agar, and it was, therefore, used in preference to glucose agar as a medium for general purposes.

A striking feature of cultures on both glucose agar and invert-sugar agar is the production of the extreme involution forms, to be described later. These are far more pronounced in the absence of blood serum. But even with blood serum added, markedly abnormal forms are still produced. And the tendency to die out seems to be associated with the production of these involution forms, as it is especially on these media that apparently vigorous growth has been followed, suddenly and without warning, by failures to grow.

Thus blood-serum glucose agar proved a far less reliable medium than blood-serum sucrose potato agar or blood-serum 25% -cane-juice peptone agar, although before the use of blood serum was introduced, glucose agar had proved itself preferable to 25% -cane-juice peptone agar and only slightly inferior to sucrose potato agar. The same remarks also apply in the case of invert-sugar agar.

On sucrose nutrient agar the organism also grows well sometimes. But it proved distinctly a less reliable medium than glucose agar. This result is surprising, in view of the converse result obtained with these sugars in potato agar, and cannot yet be explained.

For maintaining the organism in culture, an occasional change of medium, from glucose agar to sucrose potato agar and *vice versa*, proved advantageous, tending to increased vigour. Commencing before the advantages of blood serum were discovered, cultures were successfully maintained on these media without blood serum for upwards of two years, and in spite of complete failures to grow in more than half of the subcultures made. Amongst the various devices tried for encouraging growth it was found that transfers proved more reliable when, with the platinum loop containing the bacteria, a portion of the new agar was detached, pounded up and well mixed with the bacteria and some of the condensed water, the mixture being then spread over the agar surface. With enfeebled cultures, growth sometimes took place solely in the pit in the agar surface thus formed.

In bouillon the organism grew but feebly—when it grew at all. Additions of sugars were not tried.

Preparation of all the meat extract media was carried out according to the method given by Erwin F. Smith (7, Vol. 1, p. 195) for "Standard Peptonized Beef Bouillon," except that Witte's peptone was not obtainable. But the peptone used (a light cream-coloured sample prepared for bacteriological purposes) has given satisfactory results in other work, and no reason has been found to question its quality. Also, meat extract, prepared from fresh lean beef, or 0.2% Liebig's extract of meat, were each used alternatively, and were found to give similar results. Agar (shreds) was added at the rate of 2%.

Reaction of Media.—The media at first used were adjusted to +10/+15 Fuller's scale, except the potato extract media, which, without any adjustment, regularly tested +17/+18. After making some tests, a reaction of +15/+20 was adopted as the standard. The change resulted in rather fewer of the cultures failing to grow, and in the organism appearing less frequently in the long twisted filamentous forms (described later). As will be seen, the tests gave somewhat contradictory results regarding reactions between +10 and +25, but indicated that these limits should not be

exceeded either way. Further tests are required with more favourable media, now that they are known, especially those containing blood serum.

Twelve tests were made, comprising sucrose nutrient agar, adjusted to 0, +10, +20, and +25 (4 tubes of each); 50%-cane-juice peptone agar at +7, +15, +25 (4 tubes of each); sucrose potato agar at +10, +18, +25, +30, +35 (2 tubes of each); and invert-sugar potato agar at +10, +18, +25, +30, +35 (2 tubes of each). In each test, the series of 3, 4 or 5 tubes, alike except as regards reaction, were seeded from a single culture. But cultures from different isolations were compared in different series. Some of these grew more vigorously than others, but gave concordant results, as far as the varying reactions were concerned.

The potato media produced the best growth and most uniform results with fewest failures to grow (sucrose potato agar proving slightly better than invert-sugar potato agar). In every case +18 reaction (*i.e.*, without any addition of alkali or acid) proved best. Good growth also took place at both +10 and +25, but in less than half the tubes only. At +30 and +35, none or only doubtful growth occurred.

Good growth also occurred on the sucrose nutrient agar, and in every case +25 (the most acid) proved distinctly the best reaction. The vigour of growth diminished and the number of failures to grow increased as the acidity was reduced. With +0, feeble growth occurred in one tube only.

On the 50%-cane-juice peptone agar, definite growth occurred in only two of the twelve tubes. One of these was a +15 and the other a +25 tube, each belonging to a different series. Slight doubtful growth was recorded in four other tubes, six failing completely.

Optimum Temperature.—Between 25° C. and 30° C. appears to be the most favourable temperature for the incubation of the cultures. At 22° C. growth is equally good, but a little slower. The organism grows almost as well at room temperature, showing but little sensitiveness to temperature changes between 20° C. and 30° C. At 37° C. growth is slower and erratic. Most of the cultures were incubated at 25° C., but 27° C. was also used considerably.

Rate and Extent of Growth.—The organism grew slowly, cultures usually taking 1½ to 3 times as long to develop as similar cultures of *Bact. vascularum*. It is most variable, both in the time it takes to grow and the extent to which it develops on any one medium under similar conditions.

Thus on seeding blood-serum sucrose potato agar direct from the diseased cane tissues, the first signs of growth may appear in five days, isolated colonies being first distinguishable about the 7th or 8th day, and becoming large enough to pick out for subculturing about the 10th to 12th day. In other cases there may be no signs of growth for 7 to 10 days, with isolated colonies showing from 14th to 20th day. In rare instances no growth has been noticeable until as late as the 16th day. Similar rates of growth and variations have been recorded on the other media, except that the cases of slower development and weaker growth have been more numerous.

Generally speaking, the cultures which develop most rapidly ultimately produce the largest colonies. But this is not always the case. Sometimes two types of colonies, large and small, alternate in the one culture, strongly suggesting two different organisms. But it has been found that the vigorous and enfeebled types may readily be changed, the one into the other.

The most vigorous and even growth on agar is usually obtainable by seeding from a young fluid culture. On seeding from an agar culture, it is most uncertain whether vigorous, or uneven, or only feeble growth will result. Fluid media, on the other hand, are best seeded from agar cultures; they frequently fail to grow if seeded from fluid cultures. Feeble growth usually results on seeding from old cultures.

It should here be noted that the writer's cultures of *B. vascularum*, used for comparison, while agreeing closely with those described by Erwin F. Smith (7) in most respects, seemed on the whole to show somewhat greater vigour, developing both with greater rapidity and ultimately to a greater extent than he describes. Whether this was due to a more vigorous strain of the organism, or to slight variations in the media employed, is not clear. But it certainly served to allay the fears which naturally arose, that the unsatisfactory growth of the leaf-scald organism might be due to some deficiencies in the materials (*e.g.*, agar, peptone, etc.) used for preparing the media.

Isolation from the Diseased Cane Tissues.—Owing to its slowness of growth it is more than usually desirable to grow the organism from material free from contamination by foreign organisms. This may readily be secured by choosing leaf-streaks on fairly young sound leaves or tissues with reddened bundles in the upper unripe portions of stalks, and employing ordinary aseptic methods. Portions of the leaves were usually washed, dipped in alcohol, soaked three minutes in a 1/1000 solution of mercuric chloride, washed in three changes of sterile water, and then chopped and crushed up. Portions of the stem were either similarly treated, or pieces were cut out aseptically after dipping in alcohol and burning off. Juice expressed with a pair of sterile pliers from plugs of the stem obtained in this way gave excellent results.

These materials (either fragments of tissue or juice by the loopful) were usually seeded into both agar and fluid media, preferably blood-serum sucrose potato agar or blood-serum 25%-cane-juice peptone agar; and sucrose potato juice or 25%-cane-juice peptone water (the two latter either without or with blood serum).

Petri dish plate cultures were found rather unsatisfactory with this slow-growing organism, because the medium dried up too rapidly, and considerable trouble was experienced with contamination by foreign organisms. The continual preparation of fresh plates, with the addition aseptically of blood serum each time, also proved cumbersome. The use of agar slant tubes was, therefore, finally preferred, both for making isolations from the cane and for securing pure cultures. The necessary dilutions to produce isolated colonies for pure cultures were made as follows:—From a fluid culture or from a well-mixed suspension of bacteria from an agar culture a small loopful was introduced into the condensed water of the agar slant tube and well mixed. The platinum loop was resterilised and then used to smear some of the fluid over the agar surface, zig-zagging from bottom to top of the agar. The colonies towards the bottom were, of course, confluent. But with practice the dilutions were so arranged that isolated colonies were produced about the middle of the agar, becoming more and more widely separated towards the top.

The nicest isolations were obtained by making dilutions in this manner from young fluid cultures when a cloud first develops in them, i.e., 3 to 14 days after seeding with the cane tissue fragments or juice. Usually the organism is then in a vigorous condition, and will produce fairly large isolated colonies of uniform size, which may be picked out after 7-10 days. But the

fluid cultures sometimes fail to grow, the agar media being far more reliable in this respect. Isolated colonies may be obtained frequently on seeding in the agar media direct from the cane. But doubts then always arise as to whether a pure culture has actually been obtained ; and further dilution cultures, preferably from fluid media, seem desirable for this purpose.

Owing to doubts as to whether pure cultures were really being obtained, resulting from the great variability already described, dilution cultures and propagation from isolated colonies were repeated as many as six times with a number of the strains isolated. Then eight of the most divergent strains were chosen, and cultivated side by side for several months, using the same range of media for each. All were clearly one and the same organism, and the variation was thus shown to be dependent upon the different culture media employed, the age of cultures when used for reseeding and similar factors.

Appearance in Cultures.—On blood serum sucrose potato agar, fully developed colonies sometimes attain to a size of 2 mm., when the organism is in a vigorous condition. But they are more usually much smaller and frequently remain punctiform. The larger colonies are round, convex, smooth, moist, glistening (more or less) ; they appear opaque, bluish-grey, nearly white, by reflected light ; and translucent, light buff-yellow, by transmitted light. They are fairly refractive and magnified (x8) appear coarsely granular. The edges are entire. When the growth is less vigorous, the colonies are more yellow and transparent, the tiny punctiform colonies of enfeebled cultures appearing like droplets of moisture or oil on the agar surface. In streak or smear cultures the growth is usually uniform, confluent (but may be patchy), confined or very slightly spreading, more or less raised (according to vigour). The surface appears much less moist and glistening when growth is vigorous, and may become almost dry as growth develops. The colonies are usually of a soft cheesy consistency, but variable, sometimes becoming slimy, and in rare cases even rather thick and tenacious. The colour also varies considerably, from bluish-grey (almost white) to a more yellowish tint.

Its appearance on the other agar media tried, differs little from the above. On glucose agar and invert-sugar agar, the colour is a more pronounced yellow, and the consistency more inclined to be slimy and tenacious, also patchy.

Gelatine media have not yet been tried.

In sucrose potato juice a uniform cloud is first formed, which produces rolling clouds on shaking. Some cultures do not develop beyond this stage. But more usually strong film or slimy sediment formation, or both, ensue. And this may occur whether the cloud first develops in three or in twenty days, as if denoting a re-invigoration of the organism. The film formation may not advance beyond a light film-ring adhering to the sides of the test tube. Or a heavy white junket-like film of considerable thickness may form over the whole surface. It readily shakes down and diffuses through the fluid on being agitated. Similarly, the ropy-slime sediment may be slight or considerable, and may readily be diffused. Five to eight weeks are required to reach this stage, when in rare cases the fluid may also have become noticeably viscous.

With blood-serum sucrose potato juice the tendency is to form the initial cloud more rapidly, the cloud becoming denser. But subsequent growth is less, film and sediment formation being rather the exception.

In 25%-cane-juice peptone water similar growth and variations were obtained, both without and with blood serum.

In no other fluid media (except litmus milk ; see later) did growth develop beyond a more or less faint cloud.

On potato slabs, good but patchy growth may be obtained, but growth often fails. Its appearance is similar to that on the agar media, but of a variable creamy yellow colour.

Litmus Milk.—Two lots were prepared, each being obtained fresh from a different individual cow. The reactions without adjustment were slightly different to commence with, A being of a distinct lavender colour after sterilisation, while B was a blue-violet colour. Cultures from four separate isolations were each seeded into different tubes of lots A and B.

The milk became slowly but progressively more alkaline throughout ; decolourisation of the litmus, indicating reduction, commenced at the bottom of the tubes and slowly extended upwards ; this was followed by digestion of the casein, which, however, only became noticeable after several months' growth. No change was noted until 6th day, when six of the 8 tubes showed a slightly bluer tint. The 7th tube (one of lot A) showed first signs on 11th day, and the 8th (one of lot B) on 14th day. Blueing was more pronounced in lot A, but signs of reduction showed first (on 9th day) in lot B, and not until about 20th day in lot A. On 30th day, it was noted that cultures in lot A had become about as blue as the sterile control tubes of lot B, while cultures in lot B were still more blue. Subsequent reaction changes were somewhat masked by reduction, which was proceeding most rapidly about 30th day, having advanced more than half-way up each tube by the 36th day. Little further change was noticeable up to 60th day, when signs of digestion had still not been detected. However, when six months old, all the casein appeared to be digested. A heavy film, film-ring and sediment had grown, and the fluid had become opalescent and of a dirty dark colour.

For comparison, tubes were also seeded with *B. vascularum* These developed much more rapidly, considerably increased alkalinity being noticeable on 4th day. On 10th day reduction of litmus colour was nearly complete and digestion was noticeable. On 16th day strongly alkaline. On 30th day all casein appeared to be dissolved. Reaction doubtful on account of complete reduction of litmus. On 60th day they had become distinctly acid.

(*c*) Morphology.—Naming of the organism has been deferred because it has not yet been convenient to complete the study of the characters upon which its classification will be based. The following may, however, be stated :—

The organism is a slender rod with rounded ends, of variable size. In the cane tissues it occurs singly, rarely in pairs or short chains. In cultures it shows great variability, not even being constant on the one batch of medium under similar conditions. In vigorous young freshly-isolated cultures on the most favourable media (blood-serum sucrose potato agar, or blood-serum 25%-cane-juice peptone agar), it occurs characteristically in short or long curved chains, as in Fig. 20, A. Measurements recorded were as follows (averages of 10 or more in each case) :—

	Length (microns).			Breadth (microns).		
	picked large	picked small	a group average	picked large	picked small	a group average
1. *B. vasculorum* for comparison with 2 and 3 (as in Fig. 14 A)	1.8	0.9	1.0	0.5	0.3	0.4
2. Bacteria from leaf-streak crushed on slide and stained dil. carbol-fuchsin (as in Fig. 14 B) ..	1.5	0.6	1.1	0.25	0.18	0.20
3. Ditto, but from stem (as in Fig. 20 B)	1.4	0.6	0.9	0.25	0.16	0.20
4. Section leaf-blade stained carbol-fuchsin (as in Fig. 18) ..	1.1	0.5	0.7	0.23	0.12	0.18
5. Section leaf-sheath more lightly stained than No. 4 ..	0.7	0.35	0.54	0.22	0.12	0.17
6. Culture 12 days old on B.S.S.P.A. Bacteria stained dil. carbol-fuchsin on slide	1.5	0.7	1.1	0.32	0.22	0.28
7. Culture 7 days old on B.S.S. P.A. prepared as No. 6	2.1	0.7	1.3	0.28	0.20	0.24

No spores have been observed.

The organism is motile, when viewed in hanging drop preparations from suitable young vigorous cultures. Flagella staining has not been tried. It stains very feebly with methylene blue, but well with gentian violet. The most satisfactory films were obtained with carbol-fuchsin, which needs to be used slightly stronger than for ordinary bacteria (diluted about 1 in 5 or 1 in 10). It is not stained by Gram's method. The tests were made with a young agar culture, using carbol-gentian violet 4 minutes, iodine 2-4 minutes, alcohol (95%) 2-10 minutes. It was completely decolourised by 2 minutes treatment with alcohol, whereas *Staph. pyogenes albus*, used as a control, was still densely stained after 10 minutes treatment. *B. vasculorum* also proved Gram-negative by this method, although less readily decolourised than the leaf-scald organism. (Gram-positive according to E. F. Smith).

Involution forms in great variety are freely produced in cultures. While it occurs as straight rods in the cane tissues, curved forms are the rule in all but freshly isolated young vigorous cultures in the best blood-serum agar media (Fig. 21) Extraordinary forms are obtained on some media.

An extreme form, consisting of long spirally twisted irregular filaments, is shown in Fig. 22. Cultures on glucose agar and on invert-sugar agar (without blood serum) adjusted to +10/+15 reaction, frequently produced this form entirely, and especially when seeded directly from diseased cane tissues. The filaments are of variable thickness, up to 4 or 5 times that of the normal organism. In some of the less transformed cultures the filaments appear as if divided into chains of long curved rods ; but more usually no such divisions can be detected. The same form may be maintained in subcultures, but usually it becomes mixed with short " comma " and other spirillum-like forms in variable proportions. Similar, but more slender, filaments are frequently produced, associated with slow, feeble growth, in water seeded with diseased cane tissues, either solely or mixed with long and short rod forms in variable proportions, all having an attenuated appearance. Such water cultures, however, sometimes grow vigorously when nearly normal rods may alone be found in them. It is of interest to note that the first successful inoculations were made with cultures of this filamentous form in water.

Large swollen forms and other variations may also be produced. Examples of these are shown in Fig. 21. The whole gradation of forms, including short rods, long rods, much swollen forms and filaments may occur in the one culture. Spiral forms with finely tapered ends, closely resembling spirochaetes with irregular turns, are even met with occasionally.

The forms produced correspond largely to the culture medium used, but a number of subcultures may be needed before the forms characteristic of a particular medium are fully reproduced.

Next to the favourable blood-serum agar media, the least curved and most nearly normal forms are produced in good fluid media, such as sucrose potato juice and 25%-cane-juice peptone water, and on sucrose potato agar. In these, mainly slender, short and long rods are produced, occurring singly; the pairs and chains so typical of the blood serum media are rarely met with. But even here curvature and enlargement in both length and thickness are quite general, although often only slight. Spiral "snake" forms are also occasionally met with

The most swollen, large, irregular forms were regularly produced by blood-serum glucose agar (Fig. 21, B), a medium on which the organism otherwise grows well.

A peculiar grouping of the bacteria into aggregations, as represented in Fig. 23, has frequently been noted in stained preparations from cultures on most of the agar media, especially sucrose potato agar, both with and without blood serum. These groups have been found mainly in older cultures, and often associated with growth of a slimy tenacious consistency. In some cultures most of the bacteria have been grouped in this manner. The significance of this phenomenon has not been determined.

The bacterial cells degenerate and break down as the cultures get old, it being often difficult to find whole cells in cultures over a month old. The less favourable the medium the more quickly degeneration takes place.

In the earlier work these wide variations, combined with the apparent inability of isolated single cells to grow, suggested that possibly we had to do with mixed rather than pure cultures, and that failure to separate the types might be due to some symbiotic relationship or other interfering factor. However, these doubts were dissipated by the use of the blood serum agar media, which permitted the making of pure cultures by ordinary dilution methods in a normal manner. Repeated isolations from the cane with these media always produced the same type of organism, varying within narrow, well defined limits. No other type of organism could be separated from these isolations by repeatedly preparing further dilution cultures of them. Yet, even cultures thus many times purified readily reproduced the various involution forms when subcultured on other appropriate media.

(*d*) Pathogenicity.—Inoculation tests have clearly demonstrated that the organism is the cause of the disease. Two methods, viz., (1) inoculation of cuttings before planting, and (2) inoculation of embryonic leaves and stem-tissues near the apical bud with a hypodermic syringe, have proved successful. From the nine tests of these methods, detailed later, it may be gathered that either method may yield 80% to 100% positive results, provided that the requisite conditions be successfully established. But the propor-

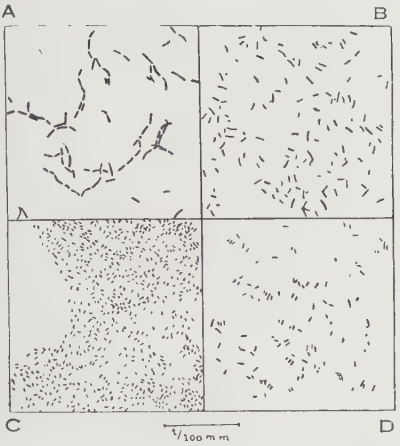


FIG. 20.—The leaf-scald organism; comparatively normal forms :—

A. In a young, newly-isolated culture on sucrose potato agar with blood serum. They occur largely in chains or pairs.

B. From diseased cane-stem tissues crushed and stained on a slide, same method as for Fig. 14.

C. As seen in a section lightly stained with carbol fuchsin.

D. As seen in a section more densely stained with carbol fuchsin.

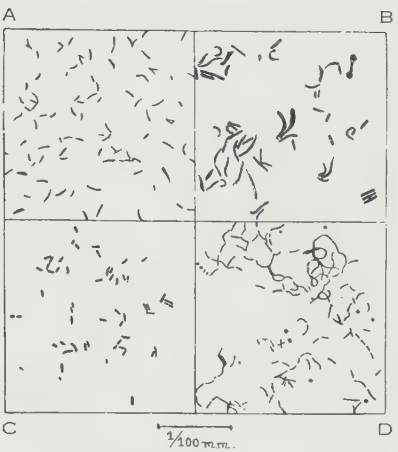


FIG. 21.—The leaf scald organism involution forms, more or less, in cultures :—

A. Subculture, five days on sucrose potato agar (without blood serum). The cells are slightly enlarged, especially thickened, and slightly curved.

B. Subculture, 14 days on blood-serum glucose agar. The cells are much enlarged, curved and distorted into fantastic shapes.

C. Subculture, 12 days on blood-serum sucrose potato agar. The cells are short, thick and slightly curved.

D. Subculture, five days on glucose agar. A great variety of forms. Curvature mostly very marked.

FIG. 22.—Leaf-scald organism, extreme involution form, in subculture five days on glucose agar, consisting of long twisted threads.

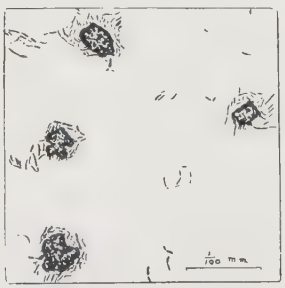


FIG. 23.—Peculiar clumping of bacteria frequently observed in stained films from agar cultures.

tion of positives may be greatly reduced by attempting to grow inoculated cuttings under too dry weather conditions. And the parasite loses its virulence on being maintained for a few months in artificial cultures.

The plants successfully inoculated reproduced all the characteristic symptoms of the disease. In certain cases, leaf-streaks and stem-tissues were sectioned, and in these the bacteria were found just as in plants naturally diseased. The organism was also re-isolated from inoculated plants, grown in pure cultures, carefully compared and used successfully for inoculating further healthy plants.

The number of successful inoculations performed under the conditions obtaining in the tests is somewhat less than might reasonably be demanded for definite proof, mainly owing to partial failure of test No. 4 (the largest of all) through unforeseen weather conditions. It is hoped to make good this deficiency when investigations are resumed, by further tests, which are also required for study of natural modes of dissemination.

Partly with the latter object in view, a variety of other methods of inoculation have also been tried. Inoculation of green leaf-blades has been tried repeatedly with negative results in all cases. The youngest partially unfolded green leaf at the apex of the stalk was most frequently selected, but older leaves were also tried. The methods included gently pricking the bacteria into the leaf with a needle, a method highly successful in the case of gumming disease ; and severely scarifying the leaf surface in order to admit the bacteria. Inoculating leaf-sheaths by pricking the bacteria in produced a slight local reaction in a few cases, a faint doubtful streak developing for several inches. In two instances only this was followed by leaf-scald developing 9-12 months later. This result was quite inconclusive, as infection from outside sources might well have occurred during this period. Inoculation by pouring several ccms. of a fluid culture around the apical spindle of developing leaves also gave similarly doubtful positives with long incubation periods in two out of five tests. Inoculation of mature stem-tissues of standing canes also yielded similar results. And root inoculation in a variety of ways consistently gave negative results. It seems unnecessary to record all these negative and inconclusive tests in greater detail here, because the failure of many of them is probably attributable to insufficient time having been allowed for the disease to develop. That well over a year should have been allowed is strongly indicated by the manner in which the disease has been observed to spread naturally in the field. It will be shown later,

in the section on dissemination, that in newly-infected canes it is apparently quite usual for the disease to remain latent for a year or more before definite symptoms of the disease become manifest.

Detailed descriptions of the nine more important tests follow (some preliminary tests, as well as the negative and inconclusive tests described above being omitted).

Inoculation of Cuttings.

1. On 23rd December, 1919, nine cuttings of Mahona were inoculated, nine similar cuttings being used as controls. Of the inoculated plants, five developed symptoms of leaf-scald within four months, the first definite leaf-streak being found on the 49th day. Two more showed symptoms up to twelve-months-old. No symptoms whatever were discovered on the eight control plants during this period (one, a butt cutting, having failed to grow). Seven (*i.e.*, 78%) of the inoculations, therefore, gave positive results.

Three stalks from a single healthy stool provided the 18 cuttings used in this trial. The alternate cuttings of each stalk were inoculated, the rest serving as controls. By inoculating the odd numbered cuttings of one stalk and the even numbered of the next, a fair division of tops, butts and other cuttings was arrived at. That the two groups of cuttings were as nearly as possible alike was thus ensured.

This system, with suitable slight modifications, was also followed in all subsequent trials.

In this trial the cuttings were inoculated by dropping on to the cut ends, and in some cases also the buds, a few drops of bacterial emulsion in sterilised rain water from subcultures on sucrose potato agar and invert-sugar agar from isolations made one month previously. (As infection of the cut ends proved effective, whether the buds were also penetrated was not clear). They were planted in the open in a fairly well-isolated experimental plot. In this, as in all such plantings, the practice has been to space the cuttings 2 ft. 6 in. or 3ft. apart in the rows, to plant the cuttings of each stalk and the stalks of each stool in rotation, except that the inoculated and control cuttings have been grouped separately, and to note the location and identity of each individual cutting on a plan, so that a separate record of each plant might be kept during its development.

2. On 28th October, 1920, 41 cuttings were inoculated, 35 cuttings being used as controls, on the same plan as in test 1. The bacteria used (eight strains from different isolations) had all been maintained 10-11 months in artificial culture, and had thus lost their virulence, although still apparently in full vigour in the five days old subcultures used. No definite leaf-scald symptoms developed in any plant, either inoculated or control, during twelve months growth, and the trial thus gave negative results. But in about 50% of the inoculated plants, slight symptoms developed between 5th and 9th months, in the form of rather indefinite spreading leaf-streaks, which were not followed by more severe manifestations. Their appearance suggested leaf-scald in a mild attenuated form.

3. On 29th September, 1921, nine cuttings were inoculated, seven being used as controls. Seven of the cuttings were inoculated on the cut ends only, the remaining two being inoculated on the buds and leaf-scars at the nodes, but not on the cut ends. A six-day-old subculture in sucrose potato juice of an isolation made three weeks previously was used as inoculum. The control

cuttings were treated in precisely the same manner as those inoculated, except that sterile sucrose potato juice was used in place of the culture. Each cutting was grown separately in a benzine tin or case. The disease rapidly developed in all nine of the inoculated plants, while all of the controls grew in a healthy manner. In the case of four cuttings, the first shoots appeared above ground with etiolated leaves within one month, and pronounced symptoms had developed in all within four months, those of which the buds were inoculated being amongst the slowest. The control plants were transplanted to the open field when 3½-months-old, and all were still healthy at 12-months-old.

Four plants of this trial are shown in Figs. 10, 11, 12.

The high proportion (100%) of positive results obtained and the severity of the symptoms exhibited were both attributed to growing the plants in pots rather than in the field, the hand watering having been so controlled that, although vigorous growth was maintained, the soil was allowed to dry out occasionally, in imitation of the conditions which, it has been found, most favour the disease in the field.

4. On 6th October, 1921, 78 cuttings were inoculated, and 64 reserved as controls. On 21st October, an additional 71 cuttings were inoculated, and 55 used as controls. One-half of each lot were planted in a rich heavy loam, and the other half in poor light sandy soil. Unusually dry weather conditions followed the planting, owing to which only about one-half of the cuttings sprouted, and these grew feebly. The dry conditions apparently also affected the development of the bacteria, for the inoculated cuttings produced nine diseased and 34 healthy plants on the rich soil; and none diseased and 39 healthy plants on the poor soil. None of the 66 control plants which grew were diseased.

Inoculation of Growing Plants.

5. On 21st October, 1921, a young growing shoot was inoculated by injecting portion of a fluid culture into the apical spindle of embryonic leaves just above the growing point. The hypodermic syringe was inserted transversely (somewhat obliquely) through the covering leaf-sheaths for this purpose. Into the control shoot, sterile water was similarly injected.

Eleven days later the leaves of both shoots had developed and unfolded sufficiently to display some of the inoculation wounds. These, in the control shoot, consisted of healthy holes, normally healed. But in the inoculated shoot, definite leaf-streaks, those typical of leaf-scald, had developed upwards and downwards directly from the inoculation holes in two leaves. The next two leaves, which unfolded a few days later, also bore inoculation holes. And from all of them leaf-streaks had developed.

These leaves matured and finally withered about seven weeks after the inoculation in the ordinary course. As the new leaves which developed in the meantime were devoid of streaks and were of normal appearance in every respect, the shoot thus regained outwardly a healthy appearance. This was of short duration, however, for one week later two more leaves bearing leaf-streaks unfolded. These were followed by many more leaves with streaks, and the plant developed all the usual symptoms of pronounced leaf-scald.

The upper portion of a single healthy stalk of Mahona, divided into two cuttings and planted on 8/8/21, each in a separate benzine tin, provided the inoculated and control plants respectively for this test. The largest primary shoot of each, similar in size and vigour, was used. The control plant remained healthy throughout until it was 12-months-old, when it was discarded It

was transplanted to the field on 17/1/22. But the inoculated plant, which at this time was already hopelessly diseased, was kept in its tin until it was discarded two or three months later.

The culture used for inoculation was a three day old subculture in blood-serum sucrose potato juice from an isolation made 11 days previously.

6. On 27th November, 1921, two young shoots of a single plant were inoculated in a similar manner to that in test 5, one shoot only of the control plant being injected with sterile water. In these cases, the inoculation evidently reached the embryonic stem tissues just below the growing point, as none of the leaves on unfolding bore inoculation wounds.

On 11th day both inoculated shoots bore characteristic leaf-streaks on their youngest unfolded green leaves. Subsequently, almost every leaf as it unfolded bore one or more leaf-streaks, one of the shoots also rapidly developing more pronounced symptoms (stunted growth, etiolation and partial withering of the leaves, etc.).

Ten weeks after the inoculation it was noted that the whole plant had become hopelessly involved, leaf-streaks having also appeared in three small shoots in addition to those inoculated, and all were much stunted in growth by comparison with the shoots of the control plant. The latter grew in a healthy manner throughout, until 12-months-old, it having been transplanted to the open field on 17/1/22.

The middle portion of a healthy stalk of Mahona divided into two cuttings and planted on 6/8/21, each in a separate benzine tin, provided the inoculated and control plants respectively.

The culture used for inoculation was a nine day old subculture in sucrose potato juice from an isolation made 26 days before. The organism in this instance was isolated from a case of leaf-scald in Malabar variety, the test having been undertaken partly with the object of establishing the identity of the disease in this highly resistant variety.

7. On 24th January, 1922, 18 large shoots were inoculated, 6 similar shoots were injected with sterile water as controls, and a number of others not treated also served as controls. The fluid cultures used were again injected in the vicinity of the growing point (above or below) with a hypodermic syringe in the same manner as in tests 5 and 6.

The first leaf-streaks were found 14 days later, on one stalk only. On 16th day three more, and on 19th day eight more stalks showed streaks. Two more were added on 28th day, 14 of the 18 inoculations thus having given positive results. The remaining four shoots did not develop symptoms within several months, and are thus to be regarded as negative. They were the four smallest of the inoculated shoots, rather lacking in vigour, owing to being overcrowded by their neighbours. The two which took 28 days to develop symptoms were also rather small shoots. That the appearance of symptoms in the smaller shoots should have been delayed, and even suppressed, is curious and cannot yet be explained.

Again, leaf-streaks were found to have developed directly from nearly every inoculation hole. But they developed equally well also when the stem tissues below the growing point were inoculated, and thus inoculation holes in the leaves did not appear.

On 27/2/22 (*i.e.*, 34 days after inoculation) two stalks were removed for dissection. In the first, the hypodermic needle had evidently been inserted

just below the growing point, several nodes having developed above the wound in the meantime. It had caused a transverse split and cavity to form in the stem tissues, but otherwise the wound had healed. Another bacterial cavity with much reddening had also developed above this one and only a half-inch below the growing point. The bacteria had spread throughout the stalk, reddened vascular bundles containing them being found in every node, but in diminishing numbers towards the butt of the stalk. Side-shoots were sprouting in characteristic fashion, particularly those nearest (above and below) the site of inoculation. Pronounced leaf-streaks were found in most of the leaf-blades and leaf-sheaths, but otherwise the leaves were green and comparatively normal.

In the second stalk, the needle had entered above the growing point, puncturing only leaf-sheaths and embryonic leaves. Pronounced leaf-streaks had developed from most of the inoculation holes in the leaf-blades, in several cases being traceable down through the leaf-sheath and into the stem. But the leaves which had developed later were devoid of leaf-streaks and apparently normal. In the stem tissues also the bacteria had developed little above the nodes bearing the punctured leaves. They had, however, spread rapidly downwards, numerous reddened vascular bundles containing them being found in every node, even in those of the underground portion of the stalk. Side-shoots had barely commenced to sprout at any point.

No sign of disease developed in any of the control stalks within three months of the inoculations, when regular inspection was discontinued. Two months later, however, two or three of them were found to have contracted leaf-scald. It had evidently spread to them from the inoculated stalks, possibly from those belonging to the same stools.

The Mahona plants used for this test consisted of several stools crowded together in a row eight feet long. No distinction between stools was made, inoculated and control shoots in many cases belonging to the one stool and growing intermingled. They were $3\frac{1}{2}$ months old when inoculated, and had made good growth. The cuttings were from a reliable source, having originally been planted as the healthy controls of an inoculation test with gumming disease.

The nine cultures used for inoculation were five day old subcultures in sucrose potato juice or in 25%-cane-juice peptone water, from three different isolations made from 3 to $3\frac{1}{2}$ months previously. They had evidently retained full virulence during this period in artificial culture.

8. On 18th February, 1922, two stalks in each of three stools in the open field were inoculated in the same manner as in tests 5, 6, and 7, two stalks in another stool being injected with sterile culture fluid as controls. Untreated stools were also left separating the treated stools. Vigorous six day old subcultures in sucrose potato juice were used, from an isolation made two years before (stool A), from an isolation made six months before (stool B), and from an isolation made one month before from the inoculated Mahona plant of test 6 (stool C).

In stool C, inoculated with the freshly isolated strain, the two stalks developed leaf-scald in the normal manner. But both the older strains had apparently lost their virulence. Faint broad ill-defined leaf-streaks had developed from the inoculation holes in stool A when the leaves unfolded, but travelled less than half the length of the leaf-blade. But four months after

inoculation both stools (A and B) again appeared healthy, whereas stool C was then severely diseased, showing etiolated leaves, stunted growth and other advanced symptoms. The control stools also remained healthy.

9. On 25th February, 1922, following the same plan as in trial 8, six further stalks were inoculated, in this case all with freshly isolated strains of the organism. Five developed leaf-scald, and thus gave positive results, while one remained apparently healthy, as did also the controls.

Of the three strains used, two were isolations from N.G. 16 variety, and the third from an inoculated Mahona plant of test No. 3.

Dissemination.

The chief modes of dissemination of the disease are by means of: (*a*) Infected planting material, (*b*) knife-infection, and (*c*) other unknown means of infection. Regarding the latter, circumstantial evidence suggests two kinds of infection, *viz.*: (1) Bud-infection, and (2) root-infection. Infection from the soil, or from any other source but living diseased cane plants, is certainly rare and unimportant, if it ever occurs.

For convenience, the planting trials, upon which these conclusions are partly based, will be described in detail after discussing the various modes of dissemination. Whereas the field observations, which do not lend themselves to similar detailed description, will be referred to during the discussion.

Ample opportunity for extensive field observations has been provided by the disease control work carried out since 1920 in the Richmond River district, as described in a previous paper (6, p. 34). This involved a systematic disease survey of the whole district twice a year, supplemented by additional examinations of individual crops according to requirements for seed selection and other purposes. The gradual spreading of the disease from field to field was thus recorded, and the origins of unexpected outbreaks in new localities were promptly investigated. Most of the unexpected outbreaks were readily explainable as being due to the agency of man, by such means as planting of cuttings from an "*unsafe*" source, or by *knife-infection* (these terms are explained later). But in many cases outbreaks occurred in which such means of dissemination could practically be excluded, and some natural agency had to be invoked for an explanation. Some unknown species of insect vectors were thus naturally suspected, as apparently the disease is liable to be carried in rare instances by some such agent over distances of at least half-a-mile, and is freely disseminated amongst healthy canes within a quarter of a mile of diseased plants.

(*a*) Dissemination with Infected Planting Material.—This has undoubtedly been the chief means of dissemination. It

has already been indicated that the disease probably reached Australia in the first place and was then distributed far and wide by means of infected cuttings. Clear evidence has been given of its introduction to the Richmond from the Clarence in 1912 in this manner. It has also been propagated on a large scale in ordinary field practice by cuttings from infected stocks being used for planting. And it is still propagated in this manner to a considerable extent, in spite of the precautions now being taken to prevent it.

Its unusually great liability to be propagated by infected cuttings is attributable to the frequent occurrence of cane, which, although infected, is of normal healthy appearance and devoid of any symptoms which betray its infected condition.

Early recognition of this fact prompted classification of fields for seed purposes as (1) safe, (2) unsafe, or (3) diseased. This was done on the basis that the discovery of a single diseased stalk sufficed to condemn the whole field as " diseased " ; while proximity (up to a quarter mile or so) to a diseased field, or being raised from doubtful seed, served to class the field as " unsafe." Only fields which appeared quite above suspicion were regarded as " safe." This drastic classification was fully justified by results, as shown (loc. cit. p. 40). Briefly, it may be said that while cuttings from " safe " fields regularly yielded healthy crops, except when planted in infected localities, those from " unsafe " fields produced diseased crops in a majority of cases, and those from " diseased " fields, diseased crops in all cases. This occurred, too, in spite of the fact that when taking cuttings from " diseased " fields, as a rule all diseased stools were carefully avoided, and only apparently healthy normal canes were used. In several cases the seed cane was even cut from a portion of the field far removed from the single point at which infection had been noted, but with a similar result.

These results show the great frequency and importance of this condition. It apparently arises from the ability of the parasite to exist in the host tissues in a latent condition for a long period, while the host plant is actively growing. As long as sufficiently uniform thrifty growth is maintained, development of the parasite may be suppressed. But the latter is apt to become active whenever its host's growth is checked, either as a result of unfavourable conditions, such as dry weather, or by approaching maturity. This is particularly liable to occur when the dormant cane buds are awakened to active growth, by preparing and planting cuttings, or by allowing the stools to ratoon after harvesting, these operations, perhaps, constituting the most severe " check " which the cane plant receives in the normal course of cultivation.

Thus, both young plant crops and young ratoon crops regularly show a marked increase in the proportion of diseased stools displaying symptoms over the crop from which they were derived. This has been a regular feature of our field observations. The extent of this increase may be well gauged from the planting trials described later, particularly from the "Seed Selection Trial" (p. 63).

Symptoms regularly develop also as the cane approaches maturity in the spring and early summer, particularly in dry seasons. In this case they follow the rather severe winter check in growth characteristic of the district, and coincide with the period of maximum sugar content, just when the plant is bursting forth into the renewed active growth which is normally maintained throughout the summer. During this period, further intermittent checks, such as are caused by deficiencies of moisture supply, appear particularly stimulating to the parasite, with consequent development of symptoms in the most pronounced forms.

Strong production of side-shoots is regularly associated with the chronic form at this season. Thus, the awakening to activity of the dormant side-buds is again seen, as in the case of cuttings planted, but in this case their activity must be attributed mainly to stimulation by the parasite. Even sound standing canes, however, are liable to exhibit some slight tendency to produce side-shoots at this season ; in extreme cases, shoots may attain a length of three or four inches towards the tops of stalks throughout whole cane fields. And Mahona is a variety particularly liable to this phenomenon. Thus, there seems to be some mutual relationship between the activity of the parasite and that of the dormant buds, each serving to stimulate the other.

As late August and September is the most favoured planting season in this district, planting usually just precedes this active development of symptoms in the spring and early summer. This arrangement increases the difficulty of selecting healthy seed cane. However, this difficulty is overcome to a large extent by the system of keeping records from year to year of the history of each field, and classifying the fields accordingly.

(*b*) Knife-Infection.—It has been shown by the "knife-infection" trials, detailed later (p. 72), that healthy cuttings are liable to become infected by being prepared with cane knives previously used on diseased canes. The inoculation tests already described also showed the high susceptibility to infection of the cut surfaces of cuttings. Cutting cane with infected cane knives is therefore a means of dissemination to be taken into account.

The wide use made of cane knives in ordinary farm practice suggests that knife-infection may be one of the chief modes of dissemination. In harvesting the crops for the factory, it would serve to transmit the disease from stool to stool in fields to be ratooned. But no harm could well result from infection of the canes sent to the factory, nor from infection of stools in fields to be ploughed out. The cane cutters might also carry infection on their knives from one field to another, the fields thus infected in certain cases being possibly in a distant locality previously free from infection. In harvesting seed cane and preparing cuttings for planting, not only would the ratoon crops be concerned, but the fields to be replanted would be still more vitally affected. The seed cane would be liable to knife-infection, both while being harvested and while being cut up into cuttings. This would apply particularly during the latter operation, because the usual practice is to transport the whole stalks in bulk to a central position, where all are cut up into cuttings. Thus, any diseased stalks included would become well distributed amongst the sound ones, and so possibly afford the inoculum for a large number.

Knife-infection thus appeared likely to offer the chief explanation of the marked increase in the proportion of diseased plants regularly observed in both young plant and young ratoon crops over that of the previous crops from which each was derived. But it was found that this was not the case in several planting trials in which precautions were taken to avoid knife-infection. In the "Seed Selection Trial" (p. 63), for example, the increase in proportion of disease was not noticeably influenced in either plant or ratoon crops by knife sterilisation. (The cane knives were immersed in boiling water for a few minutes and the chopping block surface was scorched over the naked fire before cutting each stalk into cuttings. Other possible sources of infection, *e.g.*, the hands, were disregarded as unimportant.) In this trial evidently some other mode of dissemination was responsible, infection having apparently occurred some time beforehand, and remained latent in the canes until cut and planted, or ratooned, as the case may have been.

Thus, knife-infection must be regarded as only of secondary importance from this point of view. The disease spreads so rapidly in infected areas by this other more efficient unknown means, that any additional infection by knives would scarcely matter. On the other hand, the use of infected knives for cutting in a disease-free area would be a most serious matter, particularly in the case of seed cane. And in all experimental work, constant precautions to avoid knife-infection are most necessary.

(*c*) Other Unknown Means of Infection.—Other possible means of infection by human agencies include wounds to roots by the agricultural implements used in growing crops, and wounds in the leaves and (rarely) in stems by implements, stock and workmen. No direct evidence of infection by such means has yet been obtained. As infection usually remains latent in the canes for a considerable period after it occurs, the fact that the control plants used in all the planting trials remained apparently healthy for long periods, although exposed to infection by these means, cannot be regarded as an indication that it does not occur. On the contrary, there are other reasons for regarding infection through the roots as not improbable, as will be indicated later.

However, field observations clearly indicate that the disease is also spread by some other more efficient means than these. It spreads from point to point and from field to field in a manner which strongly suggests carriage by a common flying insect.

If agricultural implements or other human agencies were responsible for the spreading in this manner, more spreading within a particular field would be expected than to neighbouring fields. But no such limitations have been observed. On the contrary, spreading still goes on in the same manner, even when it concerns neighbouring fields belonging to different farmers.

When the disease first appears in newly infected fields (*i.e.*, fields planted with safe seed cane, but within range of infection from a diseased crop), it is usual to find either a single diseased stool, or two or more widely separated from each other, near the edge of the field, less commonly towards the middle. In the succeeding ratoon crop patches of diseased stools frequently develop around these originally infected stools, "ringworm" patches, as some of the farmers have described them. They range in size from a few stools up to patches of a chain or so in diameter, in which, perhaps, 70% or 80% of the stools may be severely diseased. In addition to these patches, odd diseased stools may also be found scattered through the field. In this manner fields of Mahona usually become diseased throughout in the course of two to four years.

As regards the range of infection, it may be said that fields of Mahona within about a quarter-mile of any other diseased cane rarely survive a season without an odd stool or two becoming infected. The disease has even appeared in a few cases in fields over half-a-mile distant.

Where infection occurs in the field by the unknown means thus indicated, it usually remains latent in the plant for a period before

definite symptoms develop. As already indicated (p. 56), it appears that active development of the disease commences as the result of some check in growth to the plant, particularly one which causes the dormant side-buds to sprout. This takes place in this district, chiefly in the spring and summer, when most of these checks occur (planting, ratooning, crop maturity, etc.), even though the plant may have become infected nearly a year previously, or at any less distant time, as is strongly indicated by many of the planting trials, as well as field observations. It even seems probable that the disease may remain latent for over a year in some instances, particularly in the more resistant varieties, although no clear instances, in which the possibility of later re-infection could be definitely excluded, have yet been noted.

In many of the successful artificial inoculations, on the other hand, the period of latency was apparently eliminated, the incubation period having been quite short, viz., 11 to 19 days in the case of inoculation of growing shoots near the growing point, and one to four months in the case of cuttings.

In a few of the inoculations, however (those in which definite symptoms did not develop until a later period, after 5 to 12 months), a considerable period of latency apparently occurred. While the inoculations of mature tissues (leaves, stems, roots, dormant buds, etc.) of growing plants all gave negative or inconclusive results, probably owing to not having allowed sufficient time for the period of latency to elapse, or to not having provided a suitable " check " in the plant's growth to terminate the latency.

From these tests, it seems that the parasite finds the most favourable environment for rapid multiplication in the *actively growing* embryonic tissues, which it must reach in order to cause active development of the disease. When introduced into fully grown tissues, it is liable to remain there in a more or less dormant condition, until the natural resistance of these tissues to its development becomes broken down by some " check " of the kind already indicated. For example, it can readily penetrate the mature stem tissues of cuttings (which soon become moribund when planted), and thus rapidly reach the germinating buds. Whereas the same tissues would have proved highly resistant if, instead, the canes had been left standing and actively growing.

For the further study of this natural means of infection in the field, inoculation tests on mature organs, in which an appropriate " check " is later provided for, such as preparing and planting cuttings from the inoculated canes, are therefore needed. For

these, more effective isolation seems desirable, in order to exclude infection from outside sources, and so to allow sufficient time where needed for the period of latency to elapse.

"Bud-Infection" and "Root-Infection."—Our observations point to there being possibly two important modes of infection by natural means, which for convenience are provisionally referred to as "bud-infection" and "root-infection." Being theories which cannot yet be substantiated, their sole value is as working hypotheses.

The infection of scattered stools, and the natural spread from one field to another over considerable distances are in these hypotheses attributed to "bud-infection," probably by some flying insect.

The planting trials show that, where latent infection occurs in apparently healthy canes, usually the whole stalk is not infected, but certain cuttings of it, or it may be one cutting only, the remainder producing healthy plants. The infected cutting may be from the top or the butt or any intermediate portion of the stalk, although less top cuttings are infected than other portions. In certain cases infected cuttings have even been found alternating with the healthy ones. These features are well brought out in the plans of plots 3 and 4 of the Seed Selection Trial (Figs. 24 and 25). They strongly suggest that the latent infection is contained in individual dormant buds rather than in the stem tissues.

Further evidence of this was provided by a number of cuttings, each of which produced a diseased shoot from one bud and an apparently healthy shoot from another bud. In four instances the portion of the cutting bearing the diseased shoot was cut off, through the internode, leaving the apparently healthy shoot still growing in the field. While two of these later proved diseased, the other two produced apparently healthy stools of cane, in which no disease symptoms were found before they were harvested. As the diseased portions were not removed until six to eight weeks after the cuttings had been planted, ample time should have elapsed for the parasite to have established itself throughout the cutting, if it had been present anywhere in the stem tissues. For it does so usually in 10 to 15 days, when the cut ends of cuttings are infected just before planting. It, therefore, appears likely that the infection was confined to the dormant bud, or, at any rate, to some portion of the node bearing the bud, from which it could not readily spread through the cutting.

The hypothesis of bud-infection is also supported by study of the earliest symptoms of the chronic form, when these first appear

in one-year-old growing canes. It has already been pointed out (p. 22) that the sprouting of a single bud on the side of the stalk is sometimes the very first symptom to appear. This usually is soon followed by the sprouting of the buds immediately above and below it, on the same side of the stalk, whereas sprouting of those on the opposite side does not occur till somewhat later. Dissection of stalks displaying this phenomenon in various stages clearly indicates the bud which first sprouted as the origin of the attack. The progress of the parasite from it can be traced, by means of discoloured vascular bundles, at first upwards and downwards through several internodes on that side of the stalk. Each bud sprouts in succession, apparently, as it is reached by the parasite.

Spreading laterally is much slower, being apparently confined to the nodes, so that a longer time is required to reach any bud on the opposite side of the stalk. Great variation, however, is shown by different stalks, in both the rate and the direction of spreading. It may reach the top of the stalk before the side-shoots have had time to grow considerably, or it may travel throughout the whole length of the stalk in this manner. In other stalks spreading lengthwise is much slower, when the lateral spreading and consequent development of side-shoots on both sides becomes more pronounced.

To the hypothesis of "root-infection," on the other hand, is mainly attributed the formation of the "ring-worm" patches described above. These patches appear in some way dependent upon the intermingling of the roots of neighbouring stools during their growth. Root wounds caused by agricultural implements working between the cane rows are one of several likely sources of transmission from diseased to healthy roots. In such case, spreading laterally from one row to the next would be expected to occur equally with spreading along the rows, and this is exactly what has been noted.

Root-infection usually does not betray its presence until it suddenly manifests itself in the rapid wilting of stools already described (p. 17) as "the acute phase." Or, with plentiful rains, this phase may be more or less suppressed, being replaced by more gradual development of the chronic phase, as the parasite invades the stalks from below. Sometimes a general slight stunted growth may be noted in affected stools before any other disease symptoms appear above ground. The stalks of these stools are reduced in length, in the most marked instances to less than two-thirds that of their more normal neighbours, with corresponding reduction in

length of their internodes. Yet, in all other respects they may appear perfectly normal and healthy.

The acute phase develops as if it is entirely due to affection of the roots. Where the wilting occurs, an odd stalk or two affected by the chronic form of the disease of long standing is nearly always to be found, located as shown in Fig. 1. Such diseased stalks are less liable to wilt suddenly than the stalks of normal healthy appearance, not only those belonging to the same stool, but also those of the surrounding stools, which previously showed no evidence of the disease whatever. Whole stools frequently wilt in this manner, the wilted stalks apparently being entirely free from infection above ground. Cuttings from them, it will be shown (see p. 70, " Cuttings from Stalks Affected by the Acute Phase ") will grow into healthy stools of cane as a rule. However, the butt cuttings are frequently infected, and less frequently also the one or two cuttings next above the butt, showing the tendency of the parasite to invade the stalks from below. Their infection is usually accompanied by reddened vascular bundles and a feeble side-shoot or two, which also serve to betray their condition.

Careful examinations of the roots of wilted stools, however, have disclosed no definite evidence of root affection, but the above considerations point to these examinations having been quite inadequate.

Soil-Infection.—It has already been stated (p. 55) that " Infection from the soil, or from any other source but living diseased cane plants, is certainly rare and unimportant, if it ever occurs." This conclusion seems to be justified from the planting trials detailed on p. 73 and from our field observations. Transmission from living diseased cane plants, by the methods already indicated, would appear an adequate explanation for all the instances of infection of healthy plants hitherto observed.

Planting Trials.

1. Seed Selection Trial.—An endeavour was made to produce a healthy crop of cane, by careful selection of cuttings from a diseased field, followed by eradication of diseased stools as they appeared in the young crop.

Plot 1.

Plant Crop.—The cuttings were selected from a well-grown crop of Mahona, one-year-old plant cane, in which no material loss of crop from the disease had occurred. Rigid selection, on the basis that one diseased or suspicious stalk determined rejection of the whole stool to which it belonged, caused rejection of some 8% or 9% of stools as diseased, and a further 16% of stools as suspicious (mainly owing to a few side-shoots, or withered leaf-tips, without more definite symptoms). Stalks of small size or borer

affected were also rejected, only those of perfectly healthy appearance being used for cuttings. Precautions to avoid knife-infection were, however, not observed in this instance.

About 1,250 cuttings were planted on 19/11/19 in a plot of 0.2 acres, and these produced roughly 1,000 *stools*, the losses being mainly due to attacks of white scarabaeid grubs in the soil.

Diseased plants were first recognisable at 2-months-old, when 12 were found. Five more were found at 3-months-old, and one more at 4-months-old. After eradication of these 18 stools, no further evidence of the disease could be detected during the next three months, and it thus appeared for a time that the disease had been successfully eliminated. However, in June, 1920, a characteristic leaf-streak appeared on each of two stalks of separate stools, and these were followed by others, until at 12-months-old, 15 additional diseased stools had been thus located.

Thus the rigid selection practised failed to eliminate at least 18 infected cuttings per 1,000, probably 33 per 1,000, and, as the sequel suggests, possibly a much larger proportion.

First Ratoon Crop.—At this stage (8/11/20) the plot was harvested and ratooned, cuttings for a second planting having previously (12/10/20) been taken, as described later. In order to avoid possible knife-infection, a freshly sterilised knife was used for cutting each individual stool, the knives being sterilised by boiling for a few minutes in a can of water. Trash was burnt, and the plot cultivated in the ordinary manner. Copious rains produced a vigorous growth.

85 stools came up diseased and were eradicated on 16/12/20, five weeks after harvesting. These included the 15 diseased stools marked but not eradicated in the plant crop before harvesting. The remaining 70 must all be regarded as having also been diseased before the plant crop was harvested, although no symptoms were found in them at that time. Yet they were just as severely affected as the 15 marked stools. A further 34 diseased stools were found and removed at 2½-months-old, making a total of 119 stools (practically 12%) showing disease at this early stage. Continued rains produced such rapid growth that most of the crop lodged when 6-months-old, Although some additional diseased stools were located, thorough examination of the crop became impracticable, and further eradication had to be abandoned. At 12-months-old, when the crop was harvested and ratooned for the second time, probably 25% of stools showed the disease. But the proportion could not be determined with any degree of accuracy, owing to many canes having died through lodging and rat damage.

Second Ratoon Crop.—Owing to excessively dry weather conditions, many of the diseased stools failed to strike, but otherwise a vigorous stand was obtained. 49 diseased stools only were found and eradicated on 25/1/22 (2-months-old) after which close attention was discontinued. On the whole, however, there was undoubtedly less evidence of the disease in this crop than in the 1st ratoons.

Plot 2.

Plant Crop.—Cuttings were selected from Plot 1 on 12/10/20. In the part of the plot chosen, one stalk only was found with leaf-streaks, its stool and that on either side of it (3 stools in all) being discarded. Knife-sterilisation was strictly observed throughout the preparation of cuttings. In comparison with those used for the first planting, therefore, these cuttings ap-

peared much freer from the disease, and additional precautions were also observed in preparing them. It is worth noting here, however, that of the stools (about 100) cut for plants, the ratoons later showed 12 to be diseased.

All the cuttings (about 1,000) grew. At 6-weeks-old, 12 diseased stools were found, and at 3-months-old 4 more, all being removed. No more were found up to 7-months-old. Thus practically the same result as in the plant crop of Plot 1, was again obtained. At this stage, this crop, like the ratoon crop in Plot 1, also became very heavy and partly lodged, as a result of the copious rains, and close examination had to be discontinued. At harvesting on 7/12/21, however, a few scattered diseased stools were noted.

First Ratoon Crop.—On ratooning this plot, a full stand was obtained in spite of dry weather conditions. But growth was slow and the disease slow to develop and difficult to locate. In spite of this, 90 diseased stools were found and removed in the early stages of its growth, a result again similar to that obtained in the first ratoons of Plot 1.

Plots 3 *and* 4.

Plant Crop.—Cuttings were selected from Plot 2, on 3 and 4/10/21, again observing similar precautions to those described for Plot 2. But in this case, two plots were planted, viz.: Plot 3, adjoining Plots 1 and 2; and Plot 4 in an isolated position, with the object of eliminating the possibility of infection of the cane from adjoining plots. In addition, a record was kept of the position and origin of each cutting planted by means of the plans shown in Figs. 24 and 25, in which the stools of their origin are denoted by letters, the stalks by numbers, and the cuttings are numbered from the top downwards. Thus $\frac{T\ 2\ 3\ 4\ 5\ 6}{B\ 3}$ denotes the 6 cuttings obtained from the 3rd stalk of stool B.

Both plots suffered severely from the dry weather conditions following planting, Plot 4, located in light sandy soil, far more so than Plot 3, in rich, heavy clay-loam. Thus 46 cuttings (of 402 planted) failed in Plot 3 and 61 cuttings (of 420 planted) in Plot 4 (those marked *). And while growth was slow and the disease symptoms slow to develop in Plot 3, they were still slower in Plot 4. To this cause must be mainly attributed the lower proportion of diseased stools which developed in Plot 4, especially in the early stages of growth, rather than to its isolated position, which may have accounted for part of the difference in the later months only.

36 diseased stools in Plot 3 and 17 in Plot 4 (those marked †) were located and destroyed during the first 5 months; then 78 more in Plot 3, and 21 in Plot 4 (those marked ‡) were found at 12-months-old. Also, it is certain that a much larger proportion of the cuttings which failed were diseased than of those which grew, because about one-third of the failures in Plot 3 and two-thirds of those in Plot 4 at first produced small sickly shoots, which slowly withered and died. Although this was most suggestive of leaf-scald, it could not be definitely determined, and none were therefore counted as diseased.

The 53 stools which developed definite symptoms during the first 5 months (those marked †) may all be reasonably regarded as having developed from infected cuttings. Referring in the plans first to these, the following points may be noted :—

1. The diseased stools were scattered indiscriminately throughout both plots.

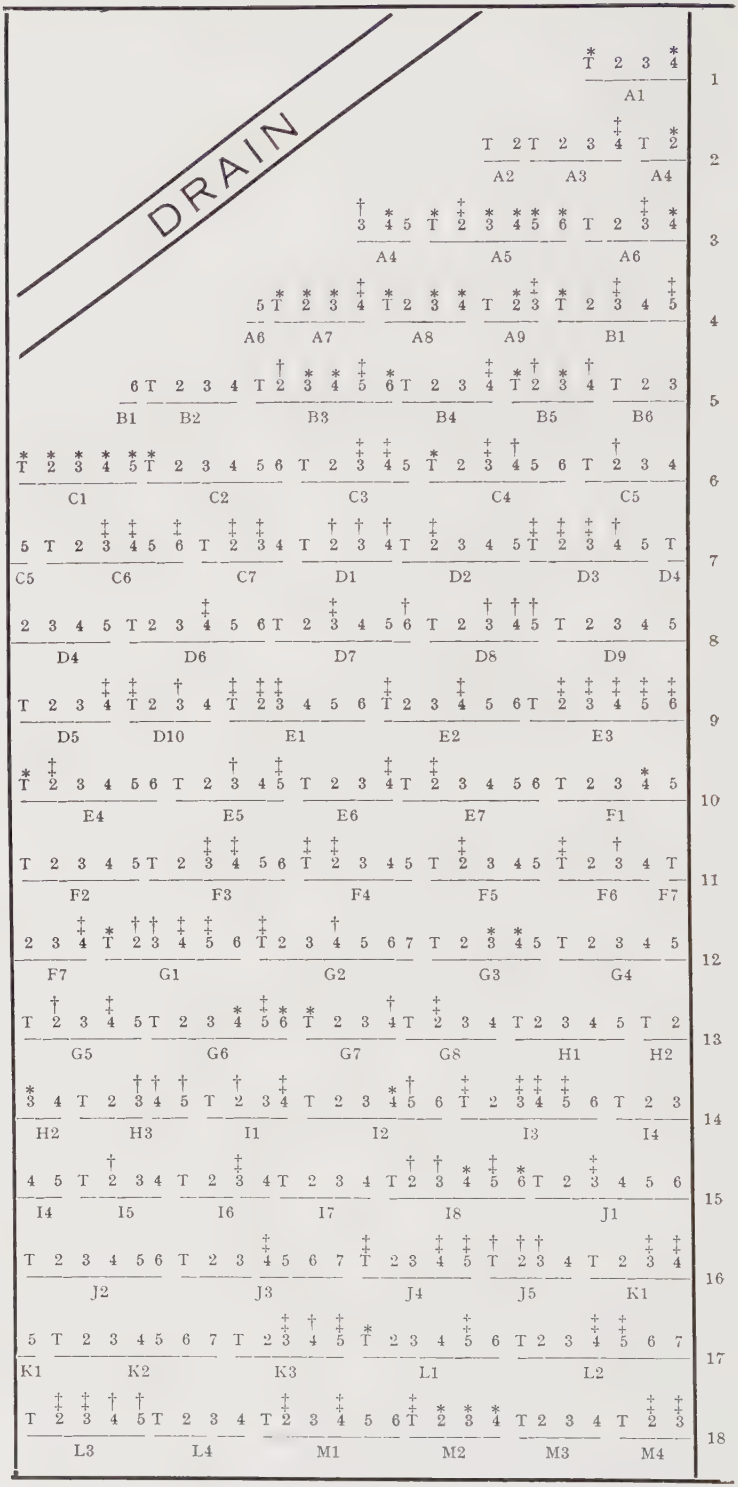


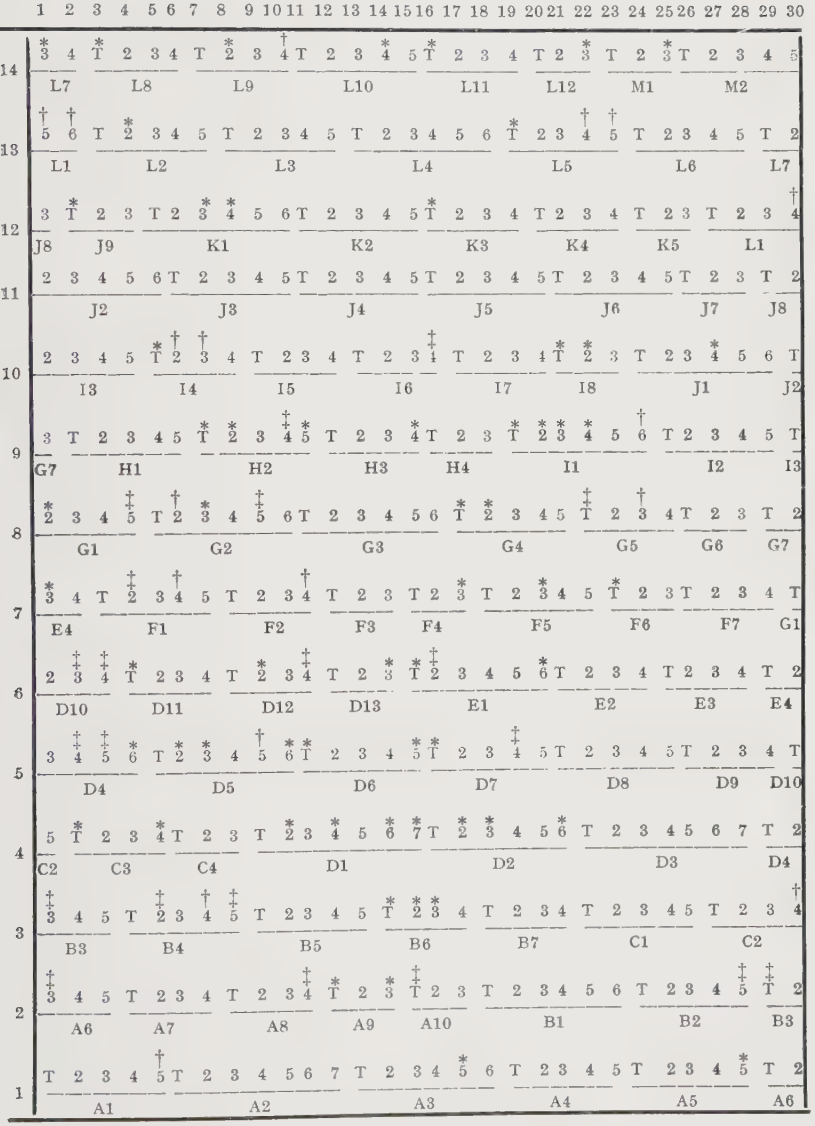
Fig. 24. SEED SELECTION TRIAL. Plan of Plot 3. (Diagrammatic)

KEY.—* denotes cutting failed.
† denotes stool developed disease symptoms before 5 months old.
‡ denotes stool developed disease symptoms between 5 and 12 months old.

Fig. 25.

SEED SELECTION TRIAL.

Plan of Plot 4. (Diagrammatic)



KEY.—* denotes cutting failed.
† denotes stool developed disease symptoms before 5 months old.
‡ denotes stool developed disease symptoms between 5 and 12 months old.

2. 12 of the 13 stools (all except stool M) cut into cuttings for Plot 3, and 8 of the 12½ stools (all except stools E, H, J, K, M) used for Plot 4, provided one or more cuttings which grew into diseased stools. The largest proportion of infected cuttings provided by a single stool was 9 out of 49 in Plot 3 (Stool D) and 6 out of 56 in Plot 4 (Stool L).

3. One of the 13 stools used for Plot 3 (Stool G) developed disease on being ratooned. It provided 5 infected cuttings.

Two of the 12½ stools used for Plot 4 (Stools B and G) developed disease on being ratooned. They provided 1 and 2 infected cuttings respectively.

Thus there was no apparent relation between the infected cuttings and the stools from which they were derived, but all the stools appeared equally liable to yield infected cuttings.

4. In 26 instances, one cutting only of the several (3 to 7 cuttings) prepared from each individual stalk proved to be infected. But pronounced grouping occurred with the 27 others, in 5 instances 3 consecutive cuttings of the one stalk proving to be infected. In no instance, however, did all the cuttings of one stalk prove to be infected.

5. The intermediate and butt cuttings proved more frequently infected than the top cuttings, of which only one was infected.

Turning now to the 99 stools which developed disease between 5 and 12-months-old (those marked ‡), their distribution strongly suggests that the majority of them also arose from infected cuttings rather than from infection during later development. For grouping according to stalks is again most pronounced, more especially in Plot 3. In one instance 5, in several instances 4, and in many instances 3 cuttings of the one stalk developed disease early or late. While diseased stools thus frequently developed next to those previously noted and eradicated, this was entirely confined (with one exception) to cases where both had originated from cuttings of the same stalk.

In other respects also their distribution resembled that of the early diseased stools described above.

Controls.—On 13th-19th October, 1920, 420 healthy Mahona cuttings from a "safe" source were planted around two sides of Plot 2, thus forming portion of the plot.

No leaf-scald appeared in these plants in the plant crop, but 10 diseased stools (2.4%) appeared and were destroyed in the young ratoons; others (not enumerated) were present when the ratoons were harvested at 12-months-old.

On 6th October, 1921, 150 healthy Mahona cuttings from a "safe" source were similarly planted alongside Plot 3, and 180 alongside Plot 4.

No leaf-scald appeared in these plants early in the plant crop, but 19 stools (13%) developed disease alongside Plot 3, and 4 stools (2%) alongside Plot 4 before the crop was harvested at 12-months-old. Plots 3 and 4 were not ratooned.

Remarks.—The controls clearly indicate that the stools which developed the disease during their first five months' growth all originated from infected cuttings, and that this probably applied up to 12-months-old in Plot 2, which grew in a wet period. But that in Plots 3 and 4 in a proportion (perhaps half) of those which showed symptoms between 5-and 12-months-old, the disease may have owed its origin to secondary infection occurring during their growth, the symptoms having appeared early as the result of dry weather conditions.

It should also be noted that diagnosis of diseased stools was based throughout upon the definite appearance of one or more of the characteristic leaf-streaks. Doubtful symptoms (stunted growth, wilting in varying degrees, etc.), were frequently noted, and caused many stools to be suspected at various stages of growth. In some of these, confirmation was secured at a later stage by the appearance of leaf-streaks, but others failed to develop more definite symptoms, and therefore had to be classed as apparently healthy.

This indicates that many diseased stools were present, besides those enumerated as such, at each inspection, not only those in which doubtful symptoms had been noted, but also others which showed no signs whatever.

2. Planting of Selected Diseased and Suspected Cuttings.—Between 25/8/19 and 17/11/19, 30 stalks in all (29 of Mahona and 1 of N.G. 16) exhibiting various symptoms were carefully selected, cut into cuttings (107 in all), and planted in a small isolated plot for observation during growth. The cuttings of each stalk were planted in rotation, the position of each being noted on the plan. Notes were then made at frequent intervals on the development of each individual cutting. Seven healthy stalks (33 cuttings) were added as controls.

Between 15/10/20 and 5/11/20, a second plot was planted on similar lines, mainly for confirmation of the more important findings of the first plot. This comprised 43 stalks (160 cuttings) of Mahona and 49 stalks (130 cuttings) of N.G. 16.

Controls were provided by the healthy Mahona cuttings planted in the vicinity as controls in other experiments, over 500 in all.

(*A*) *Cuttings from Stalks Affected by the Chronic Phase.*—These often fail to grow at all. But more usually they produce weak diseased shoots, which soon die, like those shown in Fig. 10, on the right. In some instances, the shoots, instead of curving upwards to reach the surface, grow horizontally under the soil, as if no longer negatively geotropic. Shoots of this kind were observed up to 16 inches long. They consisted of white partially unfolded leaves, much distorted and twisted in forcing their way underground.

Root development is largely suppressed with cuttings of this type. The few weak roots which sprout wither from the growing tips before attaining any considerable length.

These remarks apply similarly whether the cuttings be taken from cases of advanced disease, or from stalks showing the earliest recognisable outward symptom, *e.g.*, a leaf-streak on one leaf only, with buds not sprouted, although possibly somewhat swollen. As already explained, these symptoms indicate the stalks in which the bacteria are firmly established and actively developing.

The planting of such cuttings of Mahona is not regarded as an important factor in propagating the disease, for the reason that they usually eradicate themselves. They do not develop to any considerable size, and rarely survive four months from planting. There is danger with less sensitive varieties, however, as six of the 25 similarly diseased cuttings of N.G. 16 planted, produced stools which, although diseased and stunted, survived till harvested at 12-months-old. On being ratooned, three of these again grew with more vigour than as plant cane.

(*B*) *Cuttings from Apparently Healthy Canes in an Infected Area.*—Under this heading are included cuttings from both (*a*) *apparently healthy stalks from diseased stools*, and (*b*) *apparently healthy stools in infected blocks.* To the

latter may also be added all neighbouring blocks of susceptible varieties within range of infection, even though no trace of disease symptoms can be discovered in these blocks themselves.

Cuttings of both kinds, on being planted, behave similarly. Usually growth commences in the normal manner. A small proportion may then soon develop disease symptoms, but the majority remain apparently healthy for several months, when disease symptoms appear in a further proportion, and so on. The only difference found was a somewhat higher proportion of diseased cuttings from type (*a*) than from type (*b*). Apparently, the proportion infected diminishes more or less as distance from the source of infection is increased, but to a much less extent than might reasonably be expected.

In 1919, 17 cuttings, Mahona variety, obtained from five stalks of type (*a*) (apparently healthy stalks from diseased stools) were planted. Up to three-months-old all were comparatively normal and devoid of specific symptoms. At six months, nine stools showed definite symptoms of the disease and eight were apparently healthy. This condition was maintained up to 14-months-old, when the canes were destroyed.

In 1920, 77 cuttings, Mahona variety, from 21 stalks of the same type, yielded only six stools showing disease symptoms up to six-months-old. But of the 70 stools then apparently healthy, probably one-third proved themselves diseased before harvesting at 12-months-old. The fugitive nature of the symptoms displayed during this exceptionally wet and favourable growing period prevented more accurate determination. After ratooning, about one-half developed definite symptoms.

Also in 1920, 50 cuttings, N.G. 16 variety, from 16 stalks of this type, yielded two stools diseased and 43 apparently healthy (five cuttings having failed) up to six-months-old. It was only after ratooning that disease symptoms appeared in eight more stools of this variety.

In 1920, 35 cuttings were planted from nine stalks of the Mahona healthy control stools of the 1919 planting; also 14 cuttings from three stalks of an apparently healthy stool of the 1919 planting raised from an apparently healthy stalk from a diseased stool. These may all be regarded as stalks of type (*b*) (from apparently healthy stools in an infected plot). At three-months-old, three stools (two of the former and one of the latter) were diseased, while the remaining 46 stools remained of healthy appearance for several months after harvesting and ratooning.

The seed selection trial, already described (pp. 63-69), also consisted of the planting of stalks of type (*b*) and should be compared with the above.

(*C*) *Cuttings from Stalks Affected by the Acute Phase.*—Amongst the "wilted" stalks selected were some which had also produced side-shoots, and were thus also affected by the chronic phase. Bacteria could doubtless have been found in their internal tissues, if these had been examined. Cuttings from them, on being planted, behaved exactly like cuttings from stalks affected by the chronic phase alone, as described above, all dying within a few months.

The majority were carefully selected with buds as nearly normal in appearance as could be found. Cuttings from these behaved like those from apparently healthy stalks from diseased stools. That is to say, more than half the cuttings produced apparently healthy stools of normal appearance in every respect. This even occurred with cuttings from stalks of which every leaf was completely withered.

That a proportion of the stools thus produced were actually healthy, and not merely carrying the disease in a latent condition, was strongly indicated by harvesting and ratooning some of them, also by making further plantings from them. In the resulting stools the proportion diseased was no greater than that obtained by planting cuttings from apparently healthy stools in infected blocks, as in the seed selection trial.

Of the diseased stools produced from these " wilted " stalks, a large proportion originated from cuttings of the butt portions of the stalks. Evidently there is a tendency for the bacteria to invade these stalks from the underground parts of the plant and to travel gradually upwards. This is indicated not only by the decreased severity of the disease exhibited by consecutive cuttings of such stalks in ascending order, but also by their tendency to produce side-shoots in the same order. The extent of discolouration of the internal tissues found on examination of other similar stalks also leads to the same conclusion. A proportion of the butt cuttings thus behaved as though affected by the chronic form, and should properly have been regarded as such, although this could not be determined by the external appearance of the buds alone.

Particulars of these plantings follow :—

In 1919, one stalk, N.G. 16 variety, was selected from a stool, the whole of which had wilted through leaf-scald. That this was so was indicated by numerous side-shoots, mainly wilted, on some of the other stalks. The few live ones still remaining near the ground bore typical leaf-streaks. With the exception of a few other scattered stools in similar condition, the remainder of the block was a healthy green and in vigorous condition.

Three cuttings from the middle and upper part of this stalk (excluding the butt cutting) produced three apparently healthy stools.

In 1920, two of these stools were cut and provided 30 cuttings, all of which again produced apparently healthy stools. They were harvested and ratooned in 1921, and still no symptoms of leaf-scald were detected in them up to November, 1922.

In 1919, 9 wilted stalks, Mahona variety, were selected from diseased stools. The buds appeared normal in the upper parts of the stalks, but showed tendency to shooting towards the butts. Of the 29 cuttings from these, 7 proved apparently healthy and 22 diseased. Six of the apparently healthy ones were top cuttings, of which thus three out of nine only were diseased. Yet all from other parts of the stalks were diseased, except one—a second cutting.

In 1920, 9 similarly wilted stalks, Mahona variety, were selected. But in the case of 7 of the stalks all the buds appeared normal, the 8th had two buds at the base only, commencing to sprout, and the 9th had swollen buds on the three lower cuttings, but not on the top cutting. Of the 22 cuttings from the first seven stalks, 14 proved apparently healthy, and 8 diseased. The latter included every butt cutting and one 3rd cutting (next to the butt). Of the 8th stalk, the top and 2nd cuttings proved apparently healthy and the 3rd and butt diseased. Of the 9th, the top proved apparently healthy, and the 2nd, 3rd, and butt diseased.

Also, in 1920, 8 wilted stalks, N.G.16 variety, were selected. At planting time, wilted stalks with perfectly normal buds were unobtainable. Those selected had buds ranging from slightly swollen to 5 inches long, and the bacteria had therefore invaded all of them more or less upwards from the butt. 4 top cuttings only produced apparently healthy stools. 5 diseased stools

grew and 11 cuttings failed, in most cases dying out after producing a weak diseased shoot or two.

Controls.—From the 33 cuttings, Mahona variety, planted in 1919, one stool developed disease symptoms at 3 months, a second at 4 months, a third at 6 months, and a fourth at 14-months-old. The other 29 all remained apparently healthy. The four diseased stools all occurred in 15 of the cuttings derived from a doubtful source, viz. :—an apparently healthy block adjoining a diseased block, the cuttings having been taken from a part over 100 yards distant from the diseased block. As the other 18 cuttings, which were from a safe source, all remained apparently healthy, the diseased stools (or at any rate the first three) were attributable to infected cuttings rather than to secondary infection occurring during their growth.

From the 500 odd cuttings planted in 1920, no stool developed disease symptoms before harvesting at 12-months-old. 17 stools, however, showed symptoms in the young ratoons early in 1922, and were attributable to secondary infection taking place during the growth of the plant crop. These included the 420 control cuttings used in the seed selection trial already described.

3. Knife-infection.—The cane knives used in harvesting crops for the factory, and more particularly in harvesting seed cane and preparing cuttings for planting, were naturally suspected as a means of disseminating the disease.

The question was tested by a series of experiments, in which healthy stalks, Mahona variety, from a safe source, were cut into cuttings for planting with a cane knife with which a diseased stalk had just previously been cut into small pieces. As controls, stalks, comprising portions of the same stools as those knife-infected, were cut into cuttings with a sterilised cane knife.

On 24/1/20, of eight stalks from a single stool, four stalks (= 20 cuttings) were knife infected, and four stalks (= 17 cuttings) were planted as controls. At three-months-old, two, and at nine-months-old, five more of the stools from the infected cuttings had developed symptoms of the disease. No symptoms developed in any of the stools from the control cuttings. Two of the infected and four of the control cuttings, however, failed to grow, owing to excessively wet conditions and out of season planting. These factors adversely affected the growth of all the cuttings.

On 15/10/20, 21 stalks (= 105 cuttings) were knife-infected, and 23 stalks (= 114 cuttings) were planted as controls. At five-months-old, nine, and at 12-months-old, two more of the stools from the infected cuttings had developed the disease. No symptoms developed in any of the stools from the control cuttings during this period. After harvesting and ratooning symptoms developed in three of the knife-infected and one of the control stools, these, of course, being attributable rather to secondary infection during growth of the plant crop than to infection of the cuttings before planting.

The low proportion of positives obtained in these two trials (39% in the first and 11% in the second) was strongly suspected to be due to the weather and soil conditions obtaining in the experimental plots at that time. It was thought that these conditions suppressed the development of specific symptoms in many stools from cuttings which were actually infected at planting, and which would, if planted under conditions more favourable to the disease, have duly produced diseased stools. This opinion was based on similar indications of suppressed disease obtained in inoculation and other trials planted late in 1920.

That this opinion was well founded was shown by a number of small demonstration plots planted in widely separated localities, in which knife-infection trials of this nature (with controls) were included. The dates of planting and proportion of positives obtained in these were as follows :—

1. October, 1920, 80% positive (4 out of 5 cuttings).
2. November, 1920, 100% positive (10 out of 10 cuttings).
3. November, 1920, 40% positive (3 out of 7 cuttings).

4. Infection from the Soil.—In the plot of selected diseased and suspected cuttings planted in 1919, 31 cuttings, *viz.*, those which failed and those which produced hopelessly diseased plants, were dug out three to four months after planting (Nov.-Dec., 1919), and immediately replaced with healthy cuttings from a safe source. This was done with the object of finding whether the soil had become infected by the diseased plants and would transmit the disease to the fresh plants. The old cuttings and all the shoots from them were carefully removed, but no trouble was taken to remove the diseased roots, many of which remained in the soil around the fresh plants.

Most of the cuttings produced normal stools. But those planted close to established vigorously growing stools were somewhat stunted in growth through shading. One cutting failed to grow. All the stools were apparently healthy up to eight-months-old. At 10-to 11-months-old (October, 1920), two stools developed disease symptoms, each in one stalk only. This small proportion of diseased stools, which is similar to that found in healthy control plants grown in proximity to diseased plants at this age, was to be expected as a result of infection from neighbouring diseased stools. Infection from the soil was thus not indicated.

In the 1920 and 1921 plantings and ratoonings, stools were eradicated as soon as their symptoms permitted definite determination as leaf-scald, with the object of reducing secondary infection to a minimum. About 30 of these were replaced by transplanting a healthy growing stool of similar age into the same position. In these, a similarly small proportion of disease developed at an advanced stage only, and evidence of infection from the soil was again negative.

Also, farmers have frequently replaced diseased by healthy plants on a large scale, and replanted blocks after harvesting and ploughing out diseased crops. Extended observations in these crops have similarly indicated that healthy plants used to replace diseased ones remain apparently healthy for upwards of six months.

But for observations extending to 12 months, and beyond, into the ratoon crop, much difficulty has been experienced in obtaining conditions in which infection by natural causes from diseased plants in the vicinity could be excluded.

These conditions were fulfilled only in one small trial planted 16/9/22. In this case, two rows 8ft. long, in a well isolated position, were occupied by 11-months-old Mahona variety, well grown, although severely affected by both leaf-scald and gumming diseases. Row 2 (which had previously been used in inoculation test No. 7) contained 22 stalks definitely affected by leaf-scald, two being dead, and about as many more not showing definite symptoms. Row 1 (previously inoculated with *B. vasculorum*) also contained four stalks definitely affected by leaf-scald. The canes were cut and the stools dug out, leaving most of the roots in the soil. Healthy cuttings of Mahona, N.G. 16, and Maur. 1900 Seedling were then planted in the same positions on the same

day. Two similar rows, one alongside and the other about one chain distant, were also planted as controls, with cuttings derived from parts of the same stalks. All grew well and in a normal manner. At 14-months-old (15/11/23) all were harvested and allowed to ratoon. No trace of either leaf-scald or gumming developed in any stalk in either plant cane or the young ratoons up to 5 months-old.

7. Control Measures.

The system of control evolved in the course of these investigations has been referred to in some detail, in discussing various other aspects of the disease. A brief résumé here should, therefore, be all that is required.

While no immune varieties have been discovered, many have proved so highly resistant that little further is needed in the way of control measures, where these are grown and susceptible varieties are discarded. The degree of resistance shown by particular varieties has already been indicated on p. 16.

Where it is desired to cultivate a highly susceptible variety, such as Mahona, the most exacting precautions become necessary to ensure that no infected cuttings are planted. Owing to the fact that the disease is frequently present in a latent condition in canes of healthy appearance, it becomes necessary to avoid, for seed purposes, not only fields in which any trace of the disease is detectable, but also all fields in the vicinity (within, say, a quarter-mile or so) of diseased canes, even though no trace of the disease be detectable in them. For seed selection purposes, the former type of field is classified as "diseased," and the latter type as "unsafe." Only those fields which are entirely above suspicion may be regarded as "safe" in this connection. And, as every diseased cane provides a possible source of infection for all healthy canes within the range of infection specified, all diseased crops should be eradicated as soon as practicable. This usually means the ploughing out of "diseased" fields immediately after harvesting. Eradication of individual diseased stools proves of little value, owing to the large proportion which carry the disease in a latent condition, and are thus not detectable.

Where the disease has become so widespread that these precautions are impracticable, there remains no alternative but to replace the susceptible variety with resistant ones.

Where a moderately resistant variety, such as N.G. 16, is cultivated, some relaxation of the above exacting precautions may be allowable. For example, the use of "unsafe" seed cane for planting might be tolerated to some extent, on the basis that it would contain much fewer canes carrying the disease in a latent condition, than similarly situated fields of Mahona. Similarly, there would not

be the same urgency for the eradication of diseased crops. This has proved to be the case in both the Richmond and Clarence River districts, where large areas of N.G. 16 are still grown. Leaf-scald is now a rare occurrence in this variety, however, and has practically ceased to be of economic importance.

However, resistant varieties are liable to act as hidden carriers of infection, as they may contract the disease without becoming appreciably damaged by it, and frequently even without showing any evidence of it. Thus, any relaxation of control measures, as suggested above, is liable to lead to the disease being widely propagated in them. Sporadic cases of severe damage may then recur in them in small areas wherever the environmental conditions are particularly favourable. And a constant source of infection for any susceptible varieties will also be established.

Also, the ultimate aim of all control measures should be complete extinction of the disease. It must always be borne in mind that the greater the efficiency of the control measures, the sooner this highly desirable goal will be reached. And the nearer we approach to it, the less will be the losses sustained, and the easier and less costly control measures become.

Summary.

Leaf-scald in Australia and Fiji appears identical with the Gum-disease of Java, as described by Wilbrink (8).

The few minor differences which appear are shown, but they seem an insufficient basis for establishing the diseases as distinct, particularly seeing that the diseases were investigated in totally different cane varieties and environmental conditions generally.

The writer's investigations, being made in ignorance of Wilbrink's work, cover much of the same ground.

Wilbrink went further than the writer in the study of the organism's cultural characters, and in finding that the roots are invaded and rot.

On the other hand, the writer found evidence of additional important means of dissemination, and of the parasite's ability to exist in a latent condition in cane of healthy appearance for long periods. The control measures proposed by Wilbrink are regarded as inadequate through taking no account of these factors.

Wilbrink's comparisons show that Sereh is distinct from gum disease, the distinctions applying equally to leaf-scald.

Leaf-scald is a disease of the vascular system, closely resembling Cobb's gumming disease (*Bacterium vascularum*). They are quite distinct, however, the respective parasites differing in size and in cultural characters. The two diseases also exhibit

different symptoms in the field. Particularly the yellowish colour and viscid slime production associated with gumming are absent with leaf-scald.

That leaf-scald was introduced to Australia some time prior to the year 1900, and to Fiji a little later, is indicated by the history of Mahona, a New Guinea cane variety which should have been a most valuable commercial variety but for its susceptibility to this disease. However, its occurrence cannot be traced back with certainty beyond 1911, when it was observed as a specific disease in North Queensland.

In New South Wales, it was prevalent on the Clarence, and in 1912 was introduced from there along with N.G. 16 seed cane to the Richmond, where Mahona was the chief variety grown. Although not definitely traced, its introduction to the Tweed seems also to have occurred about this time. In both these districts it subsequently wrought great havoc, and finally caused Mahona to be discarded.

Its present distribution may be stated as practically general throughout the cane areas of Queensland and N.S.W., although it has become rare in certain districts. In Fiji it seemed to have become extinct, until fresh outbreaks occurred recently at two centres, to which it still appears to be confined.

It caused an estimated loss of 7,700 tons cane in 1,700 acres of affected Mahona which were harvested on the Richmond in the four years 1920-1923. In some badly affected fields it renders the whole crop worthless. But more usually crops are only partially destroyed. Many slightly affected crops suffer no appreciable loss.

Further losses are due to the necessity to forego the ratooning of affected crops. The sugar content of diseased canes is also seriously lowered. Possibly the most serious loss of all consists of the loss of valuable varieties, like Mahona.

Only a few highly susceptible varieties are liable to suffer so extensively as this. Moderately resistant varieties like N.G.16 suffer to a much smaller extent, while in highly resistant varieties, like Badila, fields severely damaged have been recorded only in rare instances. No immune variety has yet been found. Our present knowledge of the relative susceptibility of varieties to leaf-scald is shown in tabular form.

Leaf-scald may occur either in the form of an acute wilt, in which the plants suddenly wilt and die from no apparent cause, or in the chronic form producing dwarfed stalks with chlorotic leaves and numerous side-shoots. For many years these two forms were regarded as two distinct diseases. Originally, in Mahona, only the conspicuous wilting was observed, the comparatively inconspicuous

chronic form being overlooked. Yet the disease is usually present in a crop for a long time in the chronic form before the acute wilt appears. Then, however, many of the apparently healthy stools may suddenly wilt, the canes severely affected by the chronic form frequently remaining alive until later. This occurs chiefly in nearly mature crops in the spring.

When the stools are devoid of other symptoms, the cause of the wilting cannot be diagnosed at once, although the characteristic symptoms are usually obtainable by waiting until new shoots spring up after harvesting and ratooning. But wilting stools often have a tiny diseased shoot or two at their base, which enables definite diagnosis to be made.

The characteristic symptoms which serve for diagnosis are borne only by cane shoots affected with the chronic form, shown in all the illustrations. Fine whitish streaks on the leaves following the course of the vascular bundles are the most important symptom. Numerous side-shoots and small suckers are also produced, and white chlorotic leaves appear in advanced stages. Both stalks and leaves are stunted, the leaves being stiff and tending to curl upwards.

Reddened vascular bundles occur in the stem tissues, particularly in the nodes. Dried up cavities may also be found in advanced stages.

The leaf-streaks and the reddened vascular bundles of the stem are invaded by the bacteria, which are usually confined to the xylem vessels. But in some cases cavities are formed by the breaking down of the tissues, when the phloem and other tissues surrounding the cavity may also be invaded. This occurs commonly in the leaf-sheaths.

The reddening is accompanied by the formation of "wound-gum," which sometimes has the bacteria imbedded in it. But usually no bacteria can be detected in it.

The isolation and cultivation of the organism at first proved most difficult. But good results were secured by the use of media containing uncooked blood serum, particularly blood-serum sucrose potato agar or blood-serum 25%-cane-juice peptone agar.

The organism grows slowly in cultures, taking $1\frac{1}{2}$ to 3 times as long to develop as *B. vascularum*. On the media named, at 25° C., isolated colonies show up about 7th or 8th day, or even later.

It may readily be isolated from leaf-streaks or reddened vascular bundles of the stem and grown in pure cultures, with the aid of these media.

It displays considerable variability in cultures, particularly in vigour of growth. Its appearance in culture is described for the limited number of media on which it would grow.

It occurs in the cane tissues as a straight slender motile rod, about 1.0 μ x 0.2 μ, but in cultures tends to become somewhat larger and more or less curved. Weird involution forms, variously curved and enlarged, are produced, especially in the less favourable media. It is Gram negative, and does not form spores.

Inoculation tests with pure cultures established that it causes the disease, the plants successfully inoculated having reproduced all the characteristic symptoms, while the control plants remained healthy.

The bacteria were observed in the leaf-streaks and reddened vascular bundles in the stems of the inoculated plants, reisolated from them and successfully used for further inoculations.

Inoculation of cuttings just before planting, and injecting the bacteria into the embryonic leaves or stem tissues near the apical bud, were the most successful methods of inoculation used.

On the other hand, inoculation of matured leaf-blades, leaf-sheaths, and stem tissues of growing plants had either no effect or at most a transitory one. Root inoculation also was unsuccessful. However, there is some indication that some of these methods may succeed if a much longer time (a year or more) be allowed for the disease to develop.

Dissemination of the disease takes place in field practice (1) by the use of infected planting material; (2) by knife-infection; and (3) by some other unknown means of infection.

(1) To its carriage in infected cuttings is attributed its original introduction to Australia, and its distribution from centre to centre. It is also propagated to a serious extent by the use of infected stocks as seed cane for planting commercial crops.

The selection of healthy seed cane is rendered most difficult by the fact that infected cane is frequently of perfectly healthy normal appearance. Apparently the disease may remain for long periods in a latent condition while active growth of the host plant continues, but may become active when a check in the host's growth occurs.

Field experiences and carefully controlled planting tests have proved that any canes growing in proximity (up to a quarter-mile or so) to a diseased field are liable to be infected, and are unsafe for use as seed cane.

(2) Healthy canes are liable to become infected by being cut with knives, with which diseased canes have previously been cut.

Dissemination by knife-infection may, therefore, occur in harvesting fields to be ratooned, and even more seriously in harvesting seed cane and cutting it into sets for planting.

(3) It is evident that the disease is also widely disseminated by some other important means of infection, the nature of which is still obscure.

The distribution of the plants which become infected by these means, particularly when the disease spreads from a diseased field to an adjoining healthy field belonging to a different farmer, is strongly suggestive of the agency of flying insects.

That infection may occur by way of the dormant side-buds is suggested by the fact, established by planting trials, that one or more individual buds on a stalk are frequently infected, while all of its other buds are still healthy. Also, the dissection of newly diseased stalks has frequently indicated an individual side-bud as the original site of infection.

That infection may also occur by way of the roots is suggested by the sudden wilting of stools in the " ringworm " patches, although all of the above ground portions of many of these wilted stools are apparently free from infection. Cuttings from such wilted stalks, it has even been shown, will usually produce healthy plants, except the butt cuttings, which frequently prove to be infected.

On the other hand, no evidence of infection from the soil, or from any other source but living diseased cane plants, has been found, either in our field observations or by planting trials. If infection ever occurs by any such means, it is certainly rare and unimportant in our cane areas.

Control of the disease may readily be effected by the use of highly resistant varieties.

Where it is desired to cultivate a susceptible variety, like Mahona, the most exacting precautions are needed to secure healthy plants, owing to the fact that many canes of healthy appearance harbour the disease in a latent condition. Any cane growing within a quarter-mile or so of diseased cane, or that has been raised from seed cane from an " unsafe " source, must be regarded as " unsafe " for planting purposes. Also, all diseased crops should be ploughed out after harvesting, to get rid of sources of infection.

Where a moderately resistant variety, like N.G. 16, is concerned, the application of such measures becomes a simpler matter, owing to the slower rate of spreading of the disease in it. On the other hand, their application is seriously interfered with by the higher proportion of canes carrying the disease in a latent condition. Thus, resistant varieties are to be regarded as hidden carriers of the disease, and, as such, need special attention.

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